Thesis

Heterotrophic Microbial Colonization of the Interior of Shocked Rocks from the Haughton Impact Structure, Devon Island, Nunavut, Canadian High Arctic

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Thesis submitted in partial fulfillment of the requirements for the degree of Master of Philosophy in Polar Studies.

Scott Polar Research Institute
University of Cambridge
June 2002

Declaration

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David A. Fike

Abstract

The polar desert is one of the most extreme environments on Earth. In these regions, microorganisms have had to develop novel strategies and adaptations in order to survive. One of the most effective such strategies has been developed by microorganisms, known as endoliths, which live in the interior of rocks, escaping or mitigating the hazards of the polar desert and fully utilizing the resources available in the rock environment. The most studied groups of polar endoliths are near-surface phototrophic communities inhabiting porous sedimentary rocks in Antarctica. Here we examine a novel environment for endolithic communities: crystalline rocks that have undergone shock metamorphosis as a result of a comet or asteroid impact. Specifically, we present a characterization of the heterotrophic endolithic community and its environment in the interior of impact-shocked gneiss and breccia samples from Haughton Impact structure on Devon Island, Nunavut, in the Canadian High Arctic. The high-latitude and arid, polar climate at Haughton preclude significant populations of higher-order organisms, naturally restricting the impact structure ecosystem to microbial communities. As such, it provides a unique opportunity to examine, in a natural setting, the microbiological colonization of impact-shocked rocks. This colonization is facilitated primarily by the creation of interconnected fissures and vesicles throughout the sample, which serve as microbial habitats. Twenty-seven heterotrophic bacteria have been isolated from the samples of shocked rocks: fourteen from shocked gneiss and thirteen from breccia. Genes encoding the 16S rRNA of the isolates were sequenced to identify the isolates and characterize the community inhabiting the shocked rocks. The bacteria inhabiting the shocked gneiss and the breccia show great similarity to each other, and also to other heterotrophic communities isolated from polar environments. Scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) analysis were used together to document the *in situ* growth of these microbes, either in small groups or in large biofilms, in the interior of the samples, where they take advantage of impact-induced inhomogeneities in surface composition and inhabit cavities created by the impact-induced shock. The interiors of shocked crystalline rocks are observed to provide abundant habitats for heterotrophic bacteria, particularly as compared to unshocked samples, demonstrating, through habitat generation, the beneficial role that impact events can play in microbial ecosystems. The discovery of these heterotrophic communities within impact-shocked crystalline rocks extends our knowledge of the habitable biosphere on Earth. The colonization of the interiors of these samples has significant astrobiological applications both for considering terrestrial, microbiological contamination of meteorites from the Antarctic ice sheet and for investigating possible habitats for microbial organisms on the early Earth, and more speculatively, on Mars.

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Aerobe: Organism that can grow and metabolize in the presence of oxygen.

Autotroph: Organism whose cellular carbon is derived from inorganic carbon (CO2).

Chemotroph: Organism whose energy is derived from the oxidation of chemical compounds. Chemotrophs can be divided into two categories: chemoorganotrophs (energy derived from the oxidation of organic chemicals); chemolithotrophs (energy derived from the oxidation of inorganic chemicals).

Endolith: Organism living in the interior of a rock.

Facultative anaerobe: Aerobic organism that can also grow and metabolize in the absence of oxygen.

Gneiss: Metamorphic rock that has typically been subjected to deep burial, and exhibits a segregation of minerals into separate bands of different composition.

Heterotroph: Organism whose cellular carbon is derived from organic carbon compounds.

Meteorite: A solid mass of mineral or rock matter, derived from a comet or asteroid, but which did not completely vaporize in the Earth's atmosphere, nor upon impact with the ground.

Obligate aerobe: Organism that can only grow and metabolize in the presence of oxygen.

Obligate oligotroph: Organism adapted to low-nutrient environments. Optimal growth exhibited in low-nutrient conditions.

Oligotroph: Organism inhabiting low-nutrient environments. Optimal growth exhibited in abundant nutrient conditions.

Ornithogenic: Derived from birds (refers to soils in Antarctica).

Phototroph: Organism whose energy is derived from photochemical reactions.

Psychrophile: Organism adapted to cold environments. Optimal growth exhibited below 15°C.

Psychrotroph: Organism inhabiting cold environments. Optimal growth exhibited above 20°C.

16S rRNA: A conserved portion of the ribosomal RNA. It is used to identify organisms and determine evolutionary relationships.

16S rDNA: DNA genes encoding the 16S rRNA.

List of Abbreviations

BAS: British Antarctic Survey (National Environmental Research Council), Cambridge, UK.

EDX: Energy Dispersive X-ray Spectroscopy (also known as EDS).

GenBank: Genetic database containing 16S rDNA sequences of organisms.

GPa: Giga-Pascal = 10^9 Pascal = 10^9 N/m^2 (measure of pressure).

ICP-AES: Inductively Coupled Plasma – Atomic Absorption Spectroscopy.

PAR: Photosynthetically Available Radiation (usable by organisms for photosynthesis).

PCR: Polymerase Chain Reaction (process to amplify DNA prior to sequencing).

rDNA: ribosomal DNA (deoxyribonucleic acid).

rRNA: ribosomal RNA (ribonucleic acid).

SEM: Scanning Electron Microscopy.

SPRI: Scott Polar Research Institute, University of Cambridge, Cambridge, UK.

TSA: Tryptone Soy Agar.

UV: Ultraviolet radiation (wavelengths between 200 – 400 nm).

UVB: most dangerous category of UV radiation for organisms on the surface (280 – 320 nm).

Acknowledgments

I would like to acknowledge the support and assistance that made this research possible. Foremost, I would like to thank my supervisor Dr. Charles Cockell (British Antarctic Survey), under whose guidance this study took shape and from whom I have learned much over the past year. I would also like to thank Dr. David Pearce (British Antarctic Survey) for his work with the PCR and sequencing of the isolated bacteria, Dr. Jon Ward (British Antarctic Survey) for access to the SEM and EDX machines, and Jacqui Duffett and Dr. Sarah James for the use of the ICP-AES machine and sample preparation facilities at Royal Holloway, University of London. I am grateful to the Scott Polar Research Institute (SPRI) for providing me with support, friendship, and the opportunity to conduct this research. Finally, I would like to acknowledge the funding of my research by the Winston Churchill Foundation, and funding for the presentation of my research at the 2002 Astrobiology Science Conference at NASA Ames Research Center by the Scott Polar Research Institute and Churchill College.

Chapter 1

Introduction

Polar Desert

The polar desert is one of the least hospitable biomes on the Earth. The principle challenges faced by organisms attempting to survive in such regions are extremely low temperatures and limited nutrient availability (Wynn-William, 2000). These challenges are inescapable for organisms living in this environment. Organisms can react in two ways when faced with such environmental stresses: they can either yield to the stress conditions and make suitable provisions for survival, or attempt to resist the stress (Franks *et al.*, 1990). The organisms surviving in a polar desert have either evolved a mechanism to protect themselves from these extremes or adopted a strategy to allow for survival in spite of them (see Table 1).

The primary challenge facing organisms in a polar desert is to survive and metabolize under extremely low temperatures. Temperature extremes to which polar organisms are exposed can reach -41.2°C at Mount Fleming in Antarctica (Wynn-Williams, 2000) or –26.1°C at Haughton impact structure, Devon Island in the Arctic (Cockell *et al.*, 2002), and these temperatures can be sustained for several months during the polar winter. However, in spite of these temperatures, surviving bacterial communities have been found at Mount Fleming, where at the rock surface the annual mean temperature was –24.2°C and the minimum temperature was –41.2°C (Wynn-Williams, 2000).

The organisms that can survive in these low temperature environments may be divided into psychrotrophs and psychrophiles. Psychrotrophs have not evolved specific adaptations to the extremely low temperatures of the polar desert, and merely tolerate cold environments, where their growth is sub-optimal (Morita, 2000a). Although growth can occur around 4°C, optimal growth temperatures are between 15°C and 35°C (Bowman *et al.*, 1997). The majority of heterotrophic bacteria that have been isolated in the Arctic and Antarctica are psychrotrophs (Franzmann, 1996). These organisms have optimal growth temperatures significantly higher than the usual temperature of their environment, suggesting poor adaptation to their environment, because for optimal fitness, the maximal growth rate should occur within the environmental range of the habitat (Franzmann, 1996).

On the other hand, psychrophilic organisms thrive in and actually require cold environments (Morita, 1975). They have minimal growth temperatures less than 0°C, optimal growth temperatures below 15°C, and show no growth above 20°C (Morita, 2000a; Bowman *et al.*, 1997; Morita, 1975). Alternately, Franzmann *et al.* (1990) used the operational definition of psychrophiles as those strains that grow at 10°C, but not at 25°C. Psychrophiles have evolved specific mechanisms to cope with the thermal extremes of the polar desert (Morita, 2000a). As an example, Kappen and Friedmann (1983) found that metabolic activity among psychrophiles occurred at temperatures down to -10°C. However, at temperatures much below -10°C, the metabolism of (known) psychrophiles ceases and these microorganisms enter cryobiosis, a state of anabiosis (dormancy) resulting when ambient temperatures decrease below the minimal growth temperature (Morita, 2000a). When temperatures rise above the minimal growth temperature, these microbes resume normal function (Morita, 2000a; Nienow & Friedmann, 1993).

Examples of particular adaptations these organisms have evolved to survive and increase their productivity in these environments include: the development of enzymes capable of functioning at low temperatures; and the ability to manipulate cell membrane composition (Feller *et al.*, 1996; Nichols *et al.*, 1995; Rotert *et al.*, 1993). In order for growth in polar environments, enzymes need to possess the ability to catalyze reactions at a sufficient rate despite extremely low temperatures (Nienow & Friedmann, 1993). Psychrophilic organisms have evolved the ability to produce enzymes adapted to function at low temperatures (Feller *et al.*, 1996). These enzymes are structurally different than their counterpart enzymes in mesophilic or thermophilic organisms; enzymes isolated from psychrophilic organisms are characterized by increased structural flexibility as a result of weakened intramolecular interactions and increased enzyme-solvent interactions (Feller *et al.*, 1996). The result is that enzymes isolated from psychrophiles can catalyze reactions three times faster at temperatures around 25°C, six times faster at 10°C, and approximately ten times faster around 4°C (Feller *et al.*, 1996). The cost for these low temperature adaptations, however, is a decrease in the thermostability of these enzymes, which characteristically undergo rapid inactivation at temperatures above 30°C (Feller *et al.*, 1996).

A functional, fluid cell wall is another requirement for cell survival and growth (Rotert *et al.*, 1993). Low temperatures increase the crystalline phase of cell membranes, which is detrimental to cell function (Nichols *et al.*, 1995). Optimal growth rates can be maintained as

long as 50% of the membrane remains in a fluid state; a cell with a membrane that is less than 50% fluid has sub-optimal growth, which can be sustained until membrane fluidity decreases to 5 – 10% (Nichols *et al.*, 1995). To prevent cell membranes from entering the crystalline phase, many bacteria regulate their membrane phospholipids in response to temperature. This is achieved by manipulation of cellular fatty acid composition, increasing the proportion of lower melting-point fatty acids in response to decreased ambient temperature (Rotert *et al.*, 1993). This can involve three approaches: increasing the degree of unsaturation of individual fatty acids, and/or the overall production of unsaturated fatty acids; shortening the fatty acid chain length; and/or increasing the proportion of methyl-branched-chain fatty acids (Nichols *et al.*, 1995). While such changes can be observed phenotypically, the capacity to perform them must have evolved in the genome. The production of polyunsaturated fatty acids (PUFAs) appears to be a key physiological adaptation, which bacteria from the Antarctic appear to have evolved (Nichols *et al.*, 1995). While Kappen & Friedmann (1983) observed growth to cease at temperatures below -10°C, experiments have shown that a PUFA membrane can remain sufficiently fluid to support growth to temperatures below -20°C (Nichols *et al.*, 1995), indicating that there are perhaps 'extreme' psychrophiles, as yet undetected, with similar abilities in nature.

It is logical to believe that in a polar desert there should only be psychrophiles, since under the prevailing environmental conditions they would experience optimal growth, while psychrotrophs would endure sub-optimal growth conditions. Harder & Veldkamp (1971) demonstrated in laboratory experiments that psychrophiles out-compete psychrotrophs at low temperatures, because their growth rate is higher at these temperatures. Psychrotrophs are able to overcome this disadvantage by adopting other competitive strategies. Examples of such strategies include increasing substrate affinities, particularly in nutrient-poor environments, so that growth becomes dependent on the concentration of growth-rate-limiting substrate, and not on temperature; or developing broad tolerance for survival in fluctuating extremes, such as temperature, salinity, and relative humidity (Franzmann, 1996). The abundance of psychrotrophs in polar deserts may be explained in part by ambient temperatures that can fluctuate greatly between polar summers and winters, which specialized psychrophiles may not be able to tolerate (Franzmann, 1996).

Furthermore, the low abundance of psychrophiles (and the resulting prevalence of psychrotrophs) may be due to the fact that organisms inhabiting these environments may not

have had sufficient time for their genomes to evolve to suit the polar environment (i.e., to become 'psychrophiles'). The evolution of psychrophiles has paralleled the development of permanently cold regions on Earth, those with temperatures less than 5°C, which have only existed on Earth for the last 36 – 38 million years (Gazdzicki *et al.*, 1992, cited in Franzmann, 1996; Kennet & Shackleton, 1976). The divergence of genes from ribosomal RNA (rRNA) is observed to proceed at a rate of 1% every 25 – 50 million years (Moran *et al.*, 1993; Ochman & Wilson, 1987). Although it is not known how much of the genome must be changed to shift the minimal and optimal growth temperatures of organisms, it is presumably a significant amount and Franzmann (1996) estimates that considerable shifts in these temperatures may take millions of years. Therefore, it is likely that the observed abundance of psychrotrophs, in environments apparently more suited to psychrotrophs, may be partly due to the slow pace of evolution, as these organisms adapt to these relatively 'new' permanently cold environments.

As in all biological systems, there is no sharp discontinuity between psychophiles and psychrotrophs. In practice, there is a continuum of organisms that ranges between 'extreme' psychrophiles, those with optimal growth temperatures below 7°C, to moderate psychrophiles, to psychrotrophs, to mesophiles, and to thermophiles (Nichols *et al.*, 1995). For example, psychrotrophic Antarctic species do generally show reduced optimal growth temperatures (on the order of 10° – 20°C) when compared with their nearest non-Antarctic taxonomic counterparts (Franzmann, 1996). Although these temperatures are still higher than those for organisms deemed 'psychrophilic', this supports the suggestion that psychrotrophs are in the process of evolving to their environment, and, with time, would likely have temperature characteristics more similar to psychrophiles.

Further complications arise when using these terms, because the label 'psychrophile' or 'psychrotroph' is customarily applied based upon observations of an organism under particular environmental conditions; however, the optimal, maximal, and minimal growth temperatures for organisms are observed to vary with experimental conditions (Nichols *et al.*, 1995). For example, a decrease in water activity results in an increase to the minimum growth temperature (Nichols *et al.*, 1995). Further, salinity has been found to affect the maximum growth temperature of several psychrophilic and psychrotrophic organisms. In one case, the obligate psychrophile *Vibrio marinus* MP-1, was able to grow at 21.2°C in 3.5% salinity, but had a

maximum growth temperature of 10.5°C when the salinity was decreased to 0.7% (Nichols, *et al.*, 1995).

The second major hazard to which organisms in a polar desert are exposed is the oligotrophic (low nutrient) environment. Organisms that have successfully dealt with this challenge can be grouped into two categories: oligotrophs and obligate oligotrophs. Oligotrophs are capable of surviving and reproducing under low nutrient conditions, although these organisms have not evolved any particular adaptations to increase their fitness in the low nutrient environment (Morita, 2000b). As a result, their growth under these conditions is sub-optimal (Nienow & Friedmann, 1993). On the other hand, obligate oligotrophs have evolved mechanisms to cope with the low nutrient conditions, in which they exhibit optimal growth; however, they are unable to grow and metabolize efficiently in the presence of abundant nutrients (Hirsch *et al.*, 1988; Siebert & Hirsch, 1988; Johnston & Vestal, 1986).

While organisms have traditionally been categorized separately based upon their preferred temperature and nutrient ranges, there is evidence that these ranges are coupled (Wiebe *et al.*, 1992). In most studies of psychrophiles and cold-tolerant bacteria, substrate concentrations have been in the grams-per-liter range, and most often the temperatures used have been from 2 – 5°C (Wiebe *et al.*, 1992). However, the question of prime interest to environmental scientists is not at what temperatures or nutrient conditions can these organisms be grown in a laboratory, but what is their growth in the ranges characteristic of their natural environment (Baross & Morita, 1978). Given that, the most interesting question is whether psychrotrophic or psychrophilic organisms dominate under the actual environmental conditions.

Bacteria isolated on low-nutrient media at low-temperatures have been found to be more nutritionally versatile, although they are most often neither psychrophiles nor obligate oligotrophs, because they often grow at higher temperatures and nutrient concentrations (Wiebe *et al.* 1992; Horowitz *et al.*, 1983). Wiebe *et al.* (1992) examined growth rates over a range of substrate concentrations, where it was noted that, at 15° – 20°C, growth rates of bacteria isolated from temperatures below 0°C were independent of changes to substrate concentrations on the order of 10^4. However, in every case where the temperature was lowered, an increased substrate concentration was required for optimal growth, with the amount of additional substrate required increasing as temperatures decreased, until a maximum was reached around 4°C (Wiebe *et al.* 1992). These results demonstrate that extrapolation of temperature or nutrient preference from

experimental conditions to *in situ* conditions is, at best, problematic, and that designations of psychrophile vs. psychrotroph and obligate oligotroph vs. oligotroph have no meaning without the context of the environment in which they were observed (Wiebe *et al.* 1992).

Term	Definition
Psychrophile	Organism adapted to cold environments Optimal growth exhibited below 15°C.
Psychrotroph	Organism inhabiting cold environments. Optimal growth exhibited above 20°C.
Oligotroph	Organism inhabiting low-nutrient environments. Optimal growth exhibited in Abundant nutrient conditions.
Obligate Oligotroph	Organism adapted to low-nutrient environments. Optimal growth exhibited in Low-nutrient conditions.

Table 1: Nomenclature for Organisms in Low-Temperature and Low-Nutrient Environments.

While there are organisms, such as psychrophiles and obligate oligotrophs, which have evolved mechanisms for overcoming the particular challenges of low temperatures and limited nutrient availability, organisms in a polar desert must cope with additional environmental difficulties including frequent freeze-thaw cycles, extreme aridity, seasonally high UVB stress, abrasive winds, and a short growing season (Wynn-Williams, 2000; Nichols *et al.*, 1995). In the face of these remaining hazards, many polar microorganisms have developed a strategy that either eliminates or significantly mitigates the danger posed by these environmental conditions. One such adaptation that is widespread in the polar desert is the ability of microorganisms to occupy and exploit lithic (rock) habitats (Nienow & Friedmann, 1993).

Microorganisms that use rock as the substratum for their growth are known as lithophytes (see Figure 1; Table 2) and can be divided into three distinct categories: epilithic, hypolithic (or sublithic), or endolithic organisms (Nienow & Friedmann, 1993). Epilithic organisms live on exposed surfaces of rocks, whereas hypolithic organisms live underneath small translucent rocks that transmit sufficient light to sustain photosynthesis, albeit at reduced levels (Smith *et al.* 2000; Nienow & Friedmann, 1993). Studies of these communities reveal that both are composed primarily of cyanobacteria and lichens, although heterotrophic bacteria are also present (Smith *et al.* 2000; Wynn-Williams *et al.* 2000; Nienow & Friedmann, 1993; Vincent, 1988). Organisms in both of these groups, while utilizing some of the protective elements of the rocks, remain on the rock's exterior, and an in-depth discussion of their characteristics is beyond the scope of this study.

Endolithic organisms inhabit the interior spaces of rocks, where they can fully utilize the protection and resources available beneath the rock surface (Vincent 1988; Nienow & Friedmann, 1993; Wynn-Williams 2000). There are two main forms of endolithic organisms: cryptoendoliths and chasmoendoliths (Nienow & Friedmann, 1993). Cryptoendoliths colonize the interior pore space of rocks, whereas chasmoendoliths inhabit surface fractures in rocks. Although, in principle, these are distinct categories, in practice it is often difficult to make a clear distinction between these two forms of endolithic growth (Nienow & Friedmann, 1993), and the term endolith shall be used throughout this study.

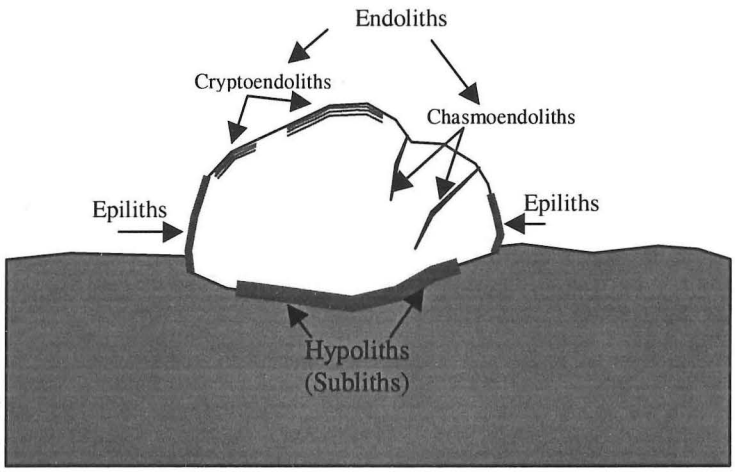

Figure 1: Lithophytic Organisms and Their Relationship with the Rock Surface.

Term	Definition
Epilith	Organism that lives on the surface of a rock
Sublithic	Organism that lives under a translucent rock
Endolith	Organism that lives in the interior of a rock
Cryptoendolith	Organism that lives within the interior pore space of a rock
Chasmoendolith	Organism that lives within cracks and crevices of a rock

Table 2: Nomenclature for Lithophytic Organisms.

Advantages of an Endolithic Existence in the Polar Desert

Rocks serve two primary functions for the endolithic organisms that inhabit them. First, they provide protection from many of the environmental extremes that threaten microbial existence in these surroundings. Secondly, the rocks act as a reservoir for water, nutrients, and heat, giving endoliths an advantage over organisms trying to survive outside the lithic environment.

The interior of a rock offers protection from several environmental hazards that otherwise severely limit microbial growth on rock surfaces, including frequent freeze-thaw cycles, seasonally high UVB stress, and abrasive winds (Wynn-Williams, 2000). Temperatures near freezing pose a real danger to organisms in the polar desert as cell walls can rupture (despite their increased flexibility) and/or the cells can be lysed by the formation of internal ice crystals (Feller *et al.*, 1996; Nienow & Friedmann, 1993). As such, rapid, usually wind-driven, temperature fluctuations can be deadly for organisms which otherwise might survive the extreme cold (Wynn-Williams, 2000; Friedmann, 1982). The interior of a rock provides protection from these fluctuations because temperatures slightly below the rock surface vary significantly less than those at the surface, affording endoliths increased protection from freeze-thaw cycles. For example, within a 42-minute period during midsummer on Linnaeus Terrace, Antarctica, the surface temperature of a rock was observed to fluctuate between –1.8°C and 5.9°C, crossing the 0°C freezing point 14 times, whereas over the same interval the temperature 3 mm beneath the rock surface remained positive, fluctuating between 1.7°C and 6.1°C (Friedmann, 1982). Endolithic organisms at or below this depth within a rock would be protected from freeze-thaw cycles. Thus, organisms that have adapted to survive within the rocks are buffered from sudden changes in temperature, drastically reducing the number of freeze-thaw cycles they undergo, which has been identified by Friedmann (1982) as one of the primary restrictions on population growth in the polar desert.

Additionally, the rocks provide endoliths with protection from excessive doses of UVB radiation and wind abrasion, two of the primary dangers that organisms on the surface must face (Cockell *et al.*, 2002; Wynn-Williams, 2000; Nienow & Friedmann, 1993). While UVB exposure is a hazard for all organisms living at the surface, its danger increases dramatically for those living in polar areas, where, during the summer months, the surface organisms are exposed to UVB continually for up to six months. Furthermore, the presence of polar ozone holes in the

atmosphere, which significantly increase the level of UVB reaching the surface, compounds the danger from UVB in polar environments.

For endolithic organisms, however, the overlying rock attenuates the UVB and organisms that are greater than 1 mm below the surface of a rock exist in an essentially UVB-free environment (the exact depth associated with this UVB protection depends on the density and translucence of the particular rock and may vary from less than 0.5 mm up to 3 mm) (Cockell *et al.*, 2002). Endolithic organisms living 0.5 mm below the rock surface receive over the course of an Arctic summer the equivalent UVB radiation dose of organisms exposed on the surface for a single day (Cockell *et al.*, 2002). Furthermore, endolithic organisms are protected from the abrasive winds that frequently scour polar deserts, particularly in the Antarctic, where katabatic winds from the continental interior, produced by the flow of cold dense air down a slope, sweep across surfaces at high speeds, essentially precluding epilithic (surface-dwelling) colonization of rocks exposed to the wind. Again, the retreat into the interior of rocks provides endolithic organisms a means of escaping from this hazard.

In addition to serving as protection from environment extremes, rocks increase the productivity of the endolithic communities they host by providing water and heat reservoirs as well as a source and reservoir for nutrients. While water is often a scarce commodity on the surface of the polar desert, the interstices and fractures of rocks serve as reservoirs to retain water. For several days following precipitation, the relative humidity in the pore spaces of a rock remains significantly higher than that of the ambient atmosphere, allowing microorganisms within the rock to metabolize and grow for longer periods of time (Nienow & Friedmann, 1993; Kappen *et al.* 1981). Kappen *et al.* (1981) report that after a snowfall on Linnaeus Terrace, Antarctica, the relative humidity within the interstitial spaces of a rock remained above 80% (sufficient to allow metabolic activity) for five days, whereas it repeatedly dropped below 20% in the ambient atmosphere over the same length of time. The water retained within rocks after precipitation can dramatically extend the growing season of endoliths, as compared to surface organisms.

Rocks provide polar endolithic organisms with a heat reservoir during the months when the sun is above the horizon. Due to their high heat capacity, rocks can reach temperatures that are significantly higher than ambient during these times. As a result of solar heating during the summer months, the surface temperature of rocks has been observed to be as much as 20°C

higher than the ambient temperature (Cockell *et al.*, 2002; Nienow & Friedmann, 1993; Vincent 1988). This extra reserve of heat allows endolithic organisms to remain active for significantly longer periods than those living on the surface (Nienow & Friedmann, 1993). Additionally, thermal radiation from the rock can melt nearby snow or ice, increasing the supply of water (and dissolved nutrients) that endoliths may access from within the rock.

A further benefit to endolithic organisms is the reserve of nutrients that exists within rocks. Nutrient availability derived from the lithic structure itself varies with rock-type and age, but Hirsch *et al.* (1988) suggest that it can be sufficient to meet the needs of an endolithic community that lacks primary producers. In addition to its nutrients content, a rock serves as a reservoir for nutrients obtained from the environment. It is estimated that nitrate precipitation from the atmosphere, approximately 21 mg N m^{-2} yr^{-1} (R. L. Mancinelli and E. I. Friedman, unpublished, cited in Nienow & Friedmann, 1993), is sufficient to ensure that endolithic communities are not nitrate limited. Furthermore, Johnston and Vestal (1986) have demonstrated no significant increase in primary productivity when a selection of Antarctic endolithic communities were exposed to increased concentrations of nitrate, ammonia, phosphate, manganese, or iron, suggesting that the concentration of these nutrients were not limiting factors on the growth in these communities. In fact, in all cases where phosphate was added to samples, the productivity decreased (Johnston & Vestal, 1986), indicating perhaps that the communities are composed of obligate oligotrophs.

Endolithic Community Structure

Within an endolithic community there are a variety of different niches filled by microorganisms with different metabolic pathways (Nienow & Friedmann, 1993; Hirsch *et al.*, 1988; Siebert & Hirsch, 1988). Two useful ways to characterize microorganisms are by the energy and carbon sources that they utilize (Egli, 2000). Microbes have developed two different systems for extracting energy from their environments: phototrophic organisms utilize light, whereas chemotrophs utilize chemicals as their energy source (Yoon *et al.*, 2000; Moat & Foster, 1995). Chemotrophs are further divided into two different categories: chemolithotrophs derive their energy from reduced inorganic chemicals; chemoorganotrophs use reduced organic chemicals as their energy source (Staley, 2000; Moat & Foster, 1995). Microbes can also be distinguished by two different systems for incorporating carbon into their cells: autotrophic

organisms are capable of converting inorganic carbon (CO_2) into organic molecules (Yoon *et al.*, 2000); heterotrophic organisms are unable to utilize CO_2 and must rely upon exterior sources of organic carbon (e.g., glucose) for their survival (Staley, 2000). The two primary constituents of endolithic communities are photoautotrophs and chemoorganoheterotrophs (Hirsch *et al.*, 1998; Siebert & Hirsch, 1988; Nienow & Friedmann, 1993), although chemolithoautotrophs may play an important role in communities lacking photoautotrophs (Hirsch *et al.*, 1988). Note that since photoheterotrophic organisms are confined to three specialized bacterial groups (green gliding bacteria, Gram-positive bacteria, and photosynthetic *Proteobacteria*), chemoorganoheterotrophs are customarily referred to simply as heterotrophs (Cavicchioli & Thomas, 2000; Staley, 2000), which will be adopted throughout this study.

Term	Definition
Autotrophy	Metabolism in which cellular carbon is derived from inorganic carbon (CO2)
Heterotrophy	Metabolism in which cellular carbon is derived from organic carbon compounds
Phototrophy	Metabolism in which energy is derived from photochemical reactions
Chemotrophy	Metabolism in which energy is derived from the oxidation of chemical compounds
Chemoorganotrophy	Metabolism in which energy is derived from the oxidation of organic chemicals
Chemolithotrophy	Metabolism in which energy is derived from the oxidation of inorganic chemicals

Table 3: Bacterial Metabolisms.

The majority of research to-date has focused on the photoautotrophs, such as lichen and cyanobacteria, which are the near-surface, photosynthetic 'primary producers' of the endolithic world (Vincent, 1988; Nienow & Friedmann, 1993). These organisms are easily detected and identified due to their large size and the distinctive pigmentation necessary for the photosynthetic process. Often these organisms appear to have an epilithic origin and have subsequently migrated into the interior of the rock, trading increased protection from temperature fluctuations, UV radiation, and desiccation for decreased photosynthetically available radiation (PAR). This has been observed, for example, in lichen that has abandoned its characteristic thallus morphology to live within the protected pore spaces of rocks (Nienow & Friedmann, 1993).

The photoautotrophic community structure is necessarily limited by the depth to which PAR can penetrate in sufficient quantities to support photosynthesis. PAR decreases by approximately

70 – 95% for each mm below the rock surface, although factors such as the presence of pigmented microorganisms and the degree of water saturation can decrease and increase, respectively, this figure by up to an order of magnitude (Nienow & Friedmann, 1993). When the PAR falls below 0.005% of its intensity at the rock surface – at a typical distance of 3 – 5 mm, photoautotrophs can no longer survive (Nienow & Friedmann, 1993). Although Nienow & Friedmann (1993) conclude that endolithic colonization is limited to this few-millimeter-deep zone below the rock surface, they have not taken into account the possibility that heterotrophic endolithic communities could exist at greater depths. This oversight may be due to the envisioned difficulty for heterotrophic organisms to survive in the interior without the photosynthetic 'primary producers' to provide them with the necessary sources of organic carbon. However, as Hirsch *et al.* (1988) noted, organics within the rock structure itself may be enough to support endoliths in the absence of primary producers. Furthermore, precipitation and snowmelt penetrating into the interior of the samples would allow the accumulation of organics from the phototrophic outer layers.

Endolithic organisms in the near-surface environment are not limited to photoautotrophs; a variety of heterotrophic bacteria and fungi are found in this region as well (Wynn-Williams, 2000; Nienow & Friedmann, 1993; Hirsch *et al.* 1988). In fact, Parker *et al.* (1977) noted that heterotrophs were by far the most numerous organisms observed in their samples from Dufek Massif, Antarctica. Wynn-Williams (2000) concludes that a large, diverse population of heterotrophic bacteria can survive in this region, either by utilizing extracellular organic chemicals produced by photoautotrophs (or possibly by chemo(litho)autotrophs) or preying directly upon them. However, as Siebert & Hirsch (1988) note, "little is known so far about heterotrophic bacteria and their part in endolithic microbial ecosystems." This paucity of knowledge is primarily due to their relatively small size and lack of distinctive pigmentation, which causes difficulties in identifying heterotrophic species, and even genera. However, the increased use and practicality of sequencing the genes encoding for 16S rRNA (16S rDNA) eliminates these constraints by allowing the detailed investigation and identification of these organisms, often to the species level, even when present in extremely low concentrations (Vincent, 2000). Molecular techniques are particularly valuable in polar environments, where the psychrophilic nature of many bacteria makes culturing them problematic (Morita 2000a) and the novelty of many organisms provides no reliable comparison against which to identify them

(Franzmann, 1996). Indeed, since the first application of phylogenetic techniques to polar endolithic communities (Colwell *et al.*, 1989), "all Antarctic strains sequenced to date have represented new species" (Franzmann, 1996). This underscores how little is understood about polar endolithic communities and their origins.

Mechanisms of Bacterial Deposition into Rocks

Despite inhabiting the interior of a rock, endolithic organisms are not completely isolated from the world outside their lithic habitat. In fact, interactions with the exterior environment are required for an endolithic community to develop, both as a means of introducing would-be endoliths into the rock interior and as a source of colonizing microorganisms. Wind, precipitation, and snowmelt serve as mechanisms that may inoculate the interior of rocks with organisms from the immediate environment (Wynn-Williams, 2000). Wind (or windblown snow) can deposit microorganisms into cracks and crevices in a rock surface, where, if motile, they may move further into the rock interior, and if nonmotile, they can be carried deeper into the rock by water or snowmelt, which deposits them while percolating through the interior pore spaces of a rock (Wynn-Williams, 2000). Soil, ice, and water from the vicinity of a given rock are the typical sources for these organisms. However, in many cases, the ultimate origin of the endolithic organisms may not be from the immediate environment of the rocks that they eventually colonize. In fact, many polar endolithic species are believed to have undergone long-distance transport prior to their entry into an endolithic community.

Various dispersal mechanisms, including atmospheric circulation, snowmelt, ocean currents, birds, fish, marine mammals, and human vectors, have been identified to transport bacteria across vast distances to the environment immediately surrounding the rock to be colonized (Vincent, 2000; Franzmann, 1996). Due to the efficacy of these dispersal mechanisms, Franzmann (1996) believes that the concept of spatial isolation is not tenable to microbes, that as a result of these dispersal mechanisms, the majority of microbes have a global distribution. Similarly, Nichols *et al.* (1995) conclude that, given the ubiquitous presence of microorganisms, the ease with which they are dispersed, and their remarkable, albeit poorly understood, survival characteristics, it is expected to find species present at widely separated geographical locations.

Of these mechanisms, atmospheric circulation is likely to be the most significant because it has the power to entrain microbes and subsequently deposit them on a global level (Vincent,

2000; Burckle & Delaney, 1999; Franzmann, 1996; Burckle & Wasell, 1995; Burckle 1995a, 1995b). Global transport of microorganisms has been supported by several studies describing bacteria that have been detected in environments that appear to be radically different from their indigenous environments, as suggested by 16S rRNA genetic analysis. Examples include an Uncultured Antarctic Ice bacterium found in hot springs at Angel Terrace, Yellowstone National Park, U. S. A. (B. Fouke, unpublished); and various thermophilic bacteria isolated on the Antarctic ice sheet (Vincent, 2000; Atlas & Bartha, 1993). Due to the temperature difference between their respective optimal temperatures and that of the environment in which they were found, it is unlikely that any of these bacteria were actually active in the environments from which they were isolated.

Additional mechanisms, such as meltwater flow and migration by birds and sea mammals are also believed to play a significant role in the transport of bacteria to, from, and among polar environments. For example, studies have demonstrated the efficacy of these mechanisms by observing the impact of Antarctic ornithogenic (derived from birds) soils on seawater bacterial microflora (Delille, 1990, 1987). A distinct difference has also been observed to exist between heterotrophic communities inhabiting sea ice and the underlying water column, between which Delille (1993) concluded, based on both physiological and taxonomic data, there exists no direct relationship. In addition to divergence resulting simply from the different natures of their respective physical environments, likely sources of divergence between these communities include the introduction of bacteria derived from avian and mammalian visitors, the run-off of glacial meltwater, and direct deposition from the atmosphere onto sea ice.

Examinations of microfossils and microorganisms within rocks exposed in Antarctica support long-distance transport of bacteria. A study of microfossils within four meteorites from the Allan Hills and Queen Alexandra Range, Antarctica has revealed the presence of modern freshwater and saltwater diatoms, as well as specimens representing extinct species (Burckle & Delaney, 1999). This indicates that both continental erosion and marine evaporation played a role in entraining microorganisms and microfossils. Opal phytoliths, microscopic particles produced by plants and released during their incineration, were also found in each meteorite (Burckle & Delaney, 1999). These data support the work of Kellogg & Kellogg (1996), which found that diatoms and opal phytoliths were transported to the Antarctic ice surface by atmospheric circulation. Freshwater, marine, and terrestrial species of diatoms have been

observed to occur from Maud Land, the McMurdo Dry Valleys, and Marie Byrd Land, Antarctica (Burckle, 1995b). From the variety of diatoms and the identification of several species present in all locations, Burckle (1995b) concluded that atmospheric processes are most likely responsible for their deposition. Another study involving igneous rocks from James Ross Island, Antarctica revealed the presence of marine, lagoonal, freshwater, and terrestrial diatoms, as well as plant fragments, pollen, and spores, within cracks and crevices in the rock surface, which were concluded to have been deposited after atmospheric transport (Burckle & Wasell, 1995).

A comparison of contamination between igneous (Marie Byrd Land, Antarctica) and sedimentary rocks (Beacon Supergroup, Antarctica) showed that both contained identical species, and that the igneous rocks had a much higher amount of microfossil deposition (Burckle, 1995a). This suggests that the surface characteristics of igneous rocks may make them more suited than sedimentary rocks for the accumulation of microfossils (and microorganisms) transported through the atmosphere. The high degree of exogenous organic matter and organisms present in Antarctic samples, despite the relative isolation of Antarctica from global patterns of atmospheric circulation (Vincent, 2000), suggests that other terrestrial environments, including the Arctic, would be exposed to more substantial nutrient and organism deposition.

A Comparison of Arctic and Antarctic Environments Containing Endoliths

Endolithic communities have been identified in terrestrial regions of both poles (Arctic: Cockell *et al.*, 2001; Cockell & Lee, 2000; Antarctic: Wynn-Williams, 2000; Nienow & Friedmann, 1993; Friedmann *et al.*, 1988; Vincent, 1988; Friedmann, 1982; Friedmann, 1977; Friedmann & Ocampo, 1976). While these communities share a polar desert climate, there are several differences in their environments. The endolithic environments from Antarctica are, for the most part, characterized by lower temperatures, higher winds, decreased precipitation, and decreased entrained nutrient deposition relative to their counterparts in Arctic endolithic communities (Nienow & Friedmann, 1993; Cockell *et al.* 2002). As such, Antarctic endoliths are subjected to a more 'extreme' environment and must be correspondingly better adapted to their environment. Although a comparative study has yet to be conducted, it is likely that the percentage of psychrophiles among Antarctic endolithic communities will be significantly higher

than amongst the Arctic endoliths, where a larger percentage may be composed of psychrotrophic organisms.

Rocks Hosting Endoliths

Since the notion of polar endoliths was first put forward (Friedmann & Ocampo, 1976), porous sedimentary rocks, such as the Beacon sandstone formation in Antarctica, have been identified as the most important for endolithic communities, because their high porosity favors colonization by endolithic bacteria (Nienow & Friedmann, 1993). While crystalline rocks, such as gneiss and granite, have also been studied in Antarctica, their typically low porosities make them poor hosts for endolithic bacteria. However, the Arctic contains a unique environment in which endolithic organisms can readily colonize crystalline rocks, specifically impact-shocked gneisses and breccias from Haughton impact structure on Devon Island in the Canadian High Arctic. Crystalline rocks, such as gneiss, can host endolithic organisms if they have undergone impact-induced shock metamorphosis, one of the many effects of an asteroid or comet impact (Cockell *et al.*, 2002; Cockell *et al.*, 2001; Dressler & Reimold, 2001; Cockell & Lee, 2000). Such impact-induced shocking can heavily fracture a rock and partially volatilize its constituent minerals, generating habitats suitable for endolithic colonization.

Meteorites

Impact-induced shock metamorphism affects the impacting body (comet or asteroid), as well as the rocks in the vicinity of the impact. Melosh (1989) showed that the shock is greatest at the point immediately below the impact point and within the impacting object itself, where pressures can exceed 100 GPa. The majority of an impacting body can be vaporized during the impact process. In cases where a sizable portion of the original impactor remains as a meteorite, it is highly shocked throughout (Dressler & Reimold, 2001; Melosh, 1989). In many cases, meteorites are covered by a fusion crust, formed during reentry or in the heat of the impact (Dressler & Reimold, 2001; Steele *et al.*, 2000). This crust, however, is frequently fractured, cracked, and weathered and serves to trap dust particles (including microfossils) that have been transported into the proximity of the meteorites either within the ice, or more likely, on the ice surface (Burckle & Delaney, 1999; Steele *et al.*, 2000). In addition to microfossils and microorganisms found on meteorite surfaces (Burckle & Delaney, 1999; Burckle & Wasell,

1995; Burckle, 1995a, 1995b), detailed microscopic investigations of the interior of the meteorite Allan Hills 84001 suggest that it may also be contaminated by endoliths (Steele *et al.*, 2000). Burckle & Delaney (1999) suggest that entrapment of micrometer-scale life forms by meteorites may be a ubiquitous process in Antarctica.

Meteorites are important because they provide physical samples of extraterrestrial materials from the solar system, yielding information concerning Solar System origin, the geochemical evolution of primitive parent objects, and the irradiation histories of material in space (Lipschutz, 1995). Of particular interest to planetary scientists are the Martian meteorites, which constitute the only *direct* source of knowledge of Martian geology, chemistry, atmospheric composition, and biology that scientists may access. The interest in these Meteorites focuses on understanding the early history of Mars, which is believed to have been similar to that of Earth, to better understand how Mars came to have its present climate, and how this can be applied to the climate stability of Earth (McKay, 1997). In recent years, much excitement has been raised by the announcement of possible relic biological activity inside of the Martian meteorite Allan Hills 84001 (McKay *et al.*, 1996). The possible origin and/or existence of life on Mars are topics of extreme scientific (and philosophical) interest. However, when investigating these questions, care must be taken to ensure the absence of terrestrial biological contamination.

Several studies have shown terrestrial surface contamination in meteorites obtained from the Antarctic ice sheet (from which Allan Hills 84001 was obtained). Burckle & Delaney (1999) conclude that terrestrial contamination, via atmospheric transport, of Antarctic meteorites is ubiquitous. Entrainment of terrestrial microfossils is believed to be the natural result of interactions between meteorites and the Antarctic (Burckle & Delaney, 1999). Microfossils and viable microorganisms have been found in several meteorites, including Allan Hills 84001 (Burckle & Delaney, 1999; Steele *et al.*, 2000). Given the high degree of impact-induced shock that these meteorites underwent, it is likely that terrestrial contamination is not limited to the surface, and that heterotrophic microbial communities could exist within the interior of these meteorites. The presence of these communities would alter the biogeochemical environment of the meteorites, possibly removing or altering any traces of relic biological activity from Mars.

Description of Present Study

To date, no work has been published identifying and characterizing the heterotrophic endolithic communities that exist significantly below the region of photosynthesis. Support for the existence of these communities has come from the work of Hirsch *et al.* (1988), who have identified near-surface, endolithic communities that appear to lack photosynthetic primary producers. A decrease in heterotrophic abundance with depth beneath the surface is expected in environments below the phototrophic zone, due to the decreased penetration of nutrients from the surface and decreased availability of nutrients associated with the lack of primary producers. However, it is also likely that, given the lack of photoautotrophs within the interior of rocks (and the resulting absence of competition), the abundance of chemoautotrophs in this environment may increase significantly. If this is the case, then the concentration of nutrients in the interior may not decrease as much as expected for rocks whose interiors are inhabited by chemoautotrophic endoliths.

The aim of the present study is to investigate possible colonization by heterotrophic bacteria of the interior of samples of impact-shocked gneiss and breccia from the ~23 Myr-old Haughton impact structure on Devon Island in the Canadian High Arctic. In particular, this study examines the origin of the possible colonizing bacteria by isolating and sequencing microorganisms present within the rock interior and comparing them with a microbial database to determine the habitat from which the isolate likely originated. Furthermore, this study aims to determine, through a combination of biological, microscopic, and spectroscopic techniques, the extent to which heterotrophic endolithic communities are present in rock interiors significantly below the photic zone. In the process, the combination of scanning electron microscopy (SEM) and X-ray energy dispersive spectroscopy will be assessed as a tool for the identification of microorganisms inhabiting impact-shocked crystalline rocks.

Chapter 2
Materials and Methodology

Study Site

Haughton Impact structure is located at 75°22'N, 89°41'W in the northwestern portion of Devon Island, Nunavut in the Canadian High Arctic (Figure 2). The impact structure is approximately 24 km in diameter (Grieve, 1988) and was created by the impact of a comet or asteroid 23.4 ± 1.0 Myr ago during the early Miocene Period (Jessberger, 1988). Furthermore, Haughton impact structure is located in a polar desert, where the paucity of organics derived from higher-order organisms makes it an ideal location for the study of microbial colonization of impact-shocked rocks and microbial interactions with the post-impact environment of a crater.

At the time of the impact, PreCambrian gneiss formed the crystalline basement on Devon Island and was overlain by 1750 m of Paleozoic sedimentary rocks (Frisch & Thorsteinsson, 1978; Robertson & Sweeney, 1983; Metzler *et al.*, 1988). These sedimentary layers were composed predominantly of dolomite and limestone, interbedded with gypsum, shale, and quartzose sandstone (Frisch & Thorsteinsson, 1978). The presence of significant amounts of gneiss, either incorporated into impact-melt breccias or as individual, impact-shocked rocks, throughout the surface of the impact structure indicates that the excavation depth for the impact exceeded 1750 m (Dressler & Reimold, 2001; Metzler *et al.*, 1988).

A survey of crystalline basement rocks by Metzler *et al.* (1988) has identified thirteen different rock types which can be grouped into eight categories: (1) sillimanite- and garnet-bearing gneiss; (2) alkali feldspar-rich aplitic or biotite-hornblende-bearing gneiss; (3) biotite and hornblende gneiss; (4) apatite-rich biotite and biotite-hornblende gneiss; (5) calcite-diopside gneiss; (6) amphibolite; (7) tonalitic orthogneiss; and (8) basalts (See Table 4). Samples of post-impact crystalline rocks have been observed to display shock metamorphism to varying degrees, ranging from pressures less than 5 GPa up to 60 GPa, with the following distribution (in GPa): 0–5: 4.5%; 10–25: 9%; 25–35: 33%; 35–45: 29%; 45–55: 18%; and 55–60: 6.5% (Metzler *et al.* 1988).

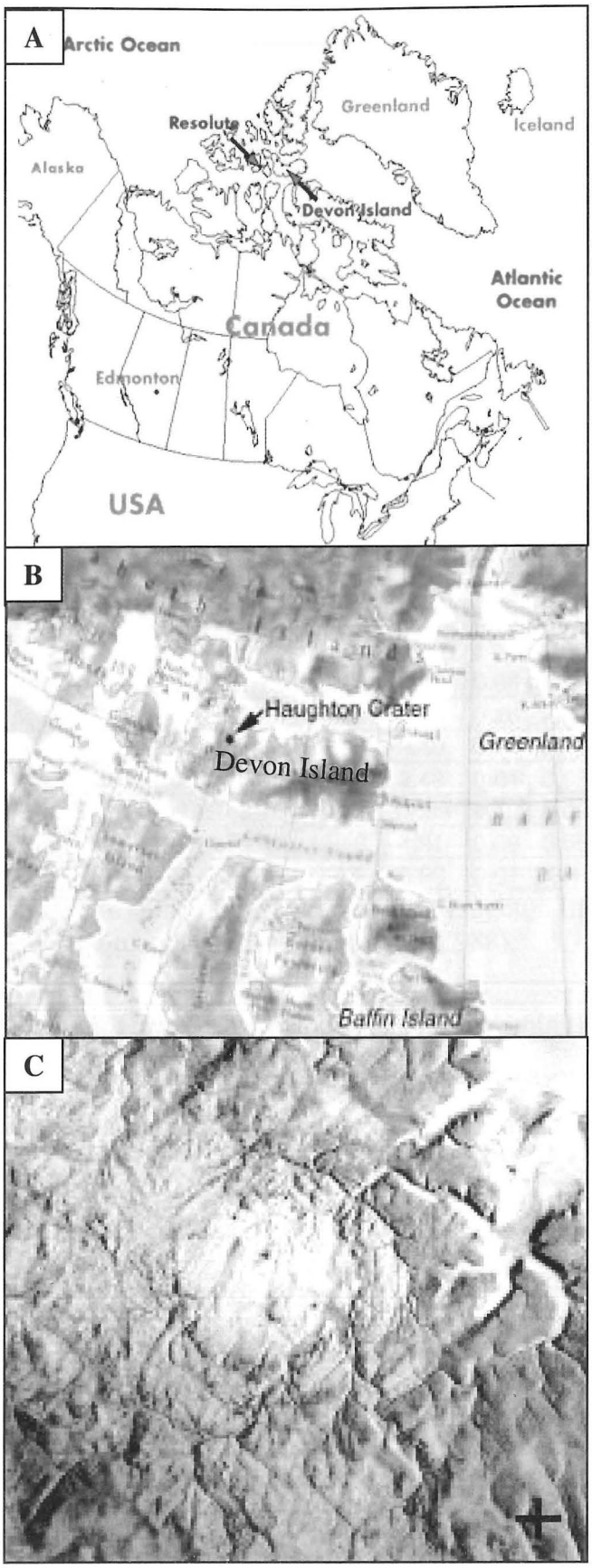

Figure 2: Geographical Location. (a) Devon Island, Nunavut, in the Canadian High Arctic. (b) Haughton impact structure is located at 75°22'N, 89°41'W in the northwestern portion of Devon Island. (c) Synthetic aperture radar image of Haughton impact structure. The crater is approximately 24 km in diameter. Image obtained from Grieve (1988).

A detailed characterization of the impact-melt breccia at Haughton has also been conducted (Osinski & Spray, 2001; Metzler *et al.*, 1988; Robertson & Sweeny, 1983) (See Table 4). The matrix of the breccia consists of two separate components: a silicate component consisting of Si-Al-Mg rich glass and a carbonate component consisting of microcrystalline calcite (Osinski & Spray, 2001). Samples of breccia typically contain up to 40% volatiles, particularly H_2O and CO_2, and have significant interior porosity (Osinski & Spray, 2001; Metzler *et al.*, 1988).

SAMPLE	Mineral Composition (% weight)										
	Si2O3	TiO2	Al2O3	Fe2O3	MnO	MgO	CaO	Na2O	K2O	P2O5	Total
Sillimanite- and Garnet-bearing Gneiss	61.56	0.57	16.12	6.26	0.11	2.2	2.08	1.081	5.69	0.08	95.8
Alkali Feldspar-rich or Biotite-Hornblende-bearing Gneiss	69.09	0.73	12.5	3.11	0.05	1.81	1.47	1.321	6.45	0.14	96.7
Biotite and Hornblende Gneiss	65.47	0.34	17.58	1.575	0.15	0.73	2.46	1.745	6.38	0.06	96.5
Apatite-rich Biotite and Biotite-Hornblende Gneiss	51.28	1.78	13.13	5.83	0.06	6.82	5.4	0.89	4.8	2.328	92.3
Calcite-Diopside Gneiss	41.06	0.35	13.00	3.69	0.26	2.80	25.00	0.55	0.95	0.02	87.68
Amphibolite	51.38	1.55	14.06	9.40	0.14	5.43	6.72	1.16	3.10	0.22	93.15
Tonalitic Orthogneiss	68.37	0.50	16.00	2.48	0.01	0.95	2.99	5.20	2.13	0.13	98.76
Basalt	48.85	3.79	12.72	12.33	0.19	6.09	8.03	1.31	0.82	0.37	94.48
Average Basement Rock	59.28	0.91	12.46	4.91	0.08	3.55	5.94	1.05	4.13	0.33	92.64
Breccia	18.47	0.14	2.93	0.93	0.01	10.10	32.87	0.06	0.78	0.05	66.34

Table 4: Mineralogy of Gneiss and Breccia from Haughton Impact Structure (adapted from Metzler *et al.*, 1988).

Climatologically, Maxwell (1981) classifies Devon Island within the climatic region IVa of the Canadian Arctic Archipelago. As such, the summer growing season is short, with an average number of 188 degree-days in July (Gold, 1988). The degree-day is a common means of assessing growth that is dependent upon temperature and/or incident solar radiation. One degree-day corresponds to one day at 1°C, while two degree-days is equivalent to either one day at 2°C or two days at 1°C each. Ecologically, the impact structure is an arid polar desert, containing less than 2% vegetation cover (Cockell et al., 2001). The soil in the Haughton impact structure is primarily dolomitic and oligotrophic (Bliss *et al.*, 1994), with bacterial numbers in the soil averaging $3 - 4 \times 10^6$ colony-forming units per gram of dry-weight soil (Cockell *et al.*, 2001). As a comparison, Antarctic soils typically contain between $10^4 - 10^9$ cells per gram of dry-weight soil and Antarctic seawater range between 10^4 and 10^6 cells/ml (Franzmann, 1996).

It has been reported (Cockell *et al.*, 2002) that heavily shocked gneisses (those subject to stresses above 30 GPa) from the Haughton impact structure are preferentially colonized by

photosynthetic endoliths relative to lesser-shocked (< 5 GPa) or unshocked gneiss. Heavily shocked samples were found colonized in approximately 73% of the cases (22 out of 30 samples), whereas weakly or un-shocked samples were colonized only 3% of the time (1 out of 30 samples), and this colonization was limited to the outer weathering crust of the sample (Cockell *et al.*, 2002). The predominant genus identified within these communities appears to be the coccoid cyanobacteria *Chroococcidiopsis*, which is typically associated with polar endolithic communities, and has been identified from the soil around Haughton (Cockell *et al.*, 2001; Nienow & Friedmann, 1993).

Several reasons have been identified for the disparity between the phototrophic colonization of the unshocked and shocked gneiss (Cockell *et al.*, 2002; Cockell & Lee, 2000). There were clear differences in the physical properties of the samples of unshocked gneiss and those of the shocked gneiss (see Table 5; Figure 3). The impact-induced shocking decreased the average density of the gneiss samples from 2.61 ± 0.17 g/cm^3 to 1.17 ± 0.24 g/cm^3. A corresponding increase was observed in the surface area of interior pore spaces with a diameter greater than 1 μm (i.e., large enough to host a microorganism) from 0.004 ± 0.0035 m^2/g in weakly shocked samples to 0.10 ± 0.057 m^2/g in highly shocked samples. Further, post-impact optical transmission increased by more than an order of magnitude, with transmitted intensities 0.5 mm below the surface increasing from 1.8% in the unshocked samples to 22% in the shocked samples (Cockell *et al.*, 2002). This corresponds to an increase in the maximum depth at which photosynthesis could occur from 1.3 mm to 3.6 mm (Cockell *et al.*, 2002). This increase is due to the increased translucence of the rock, caused by preferential volatilization of dark minerals (such as amphiboles), as well as an increase in the penetration distance into the rock through which the PAR can pass before it is attenuated, caused by the decreased post-impact density.

Property	Shocked Rock (>20 GPa)	Low-Shock Rock (<10GPa)
[1]Density (g·cm^{-3})	1.17 ± 0.24	2.61 ± 0.17
[2]Porosity (m^2·g^{-1})	0.10 ± 0.057	0.004 ± 0.0035
[3]Light Transmission (% incident)	0.22 ± 0.02	0.02 ± 0.01

Table 5: Physical Parameters of Unshocked and Shocked Gneiss from Haughton impact structure. [1]Values are the mean of three samples. [2]Total pore area (m^2) per cm^3 of sample for pores ≥ 1μm. Values are the mean of three samples. [3]Mean percentage of incident light at 680 nm transmitted through 0.5 mm (adapted from Cockell *et al.*, 2002).

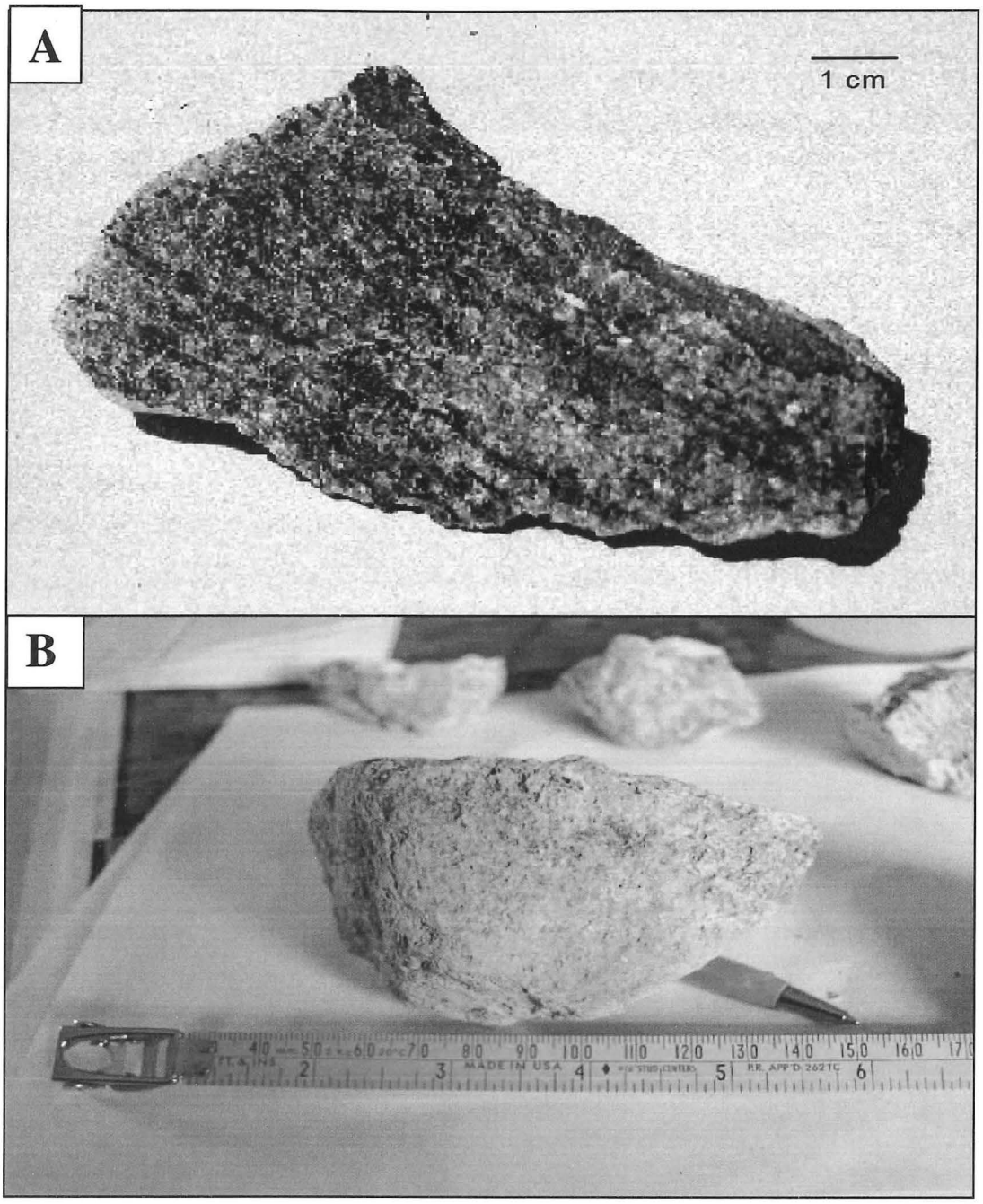

Figure 3: Images of gneiss from the Haughton impact crater. (a) Sample of unshocked (< 5GPa) gneiss. (b) Sample of shocked (25 – 45 GPa) gneiss. Note the high porosity and relative absence of dark minerals in the shocked sample.

Although the lesser concentration of PAR that penetrates the unshocked gneiss contributes to the decrease in its colonization rate relative to shocked gneiss, it is unlikely that this attenuation of available PAR could explain the disparity between the frequency of phototrophic colonization of unshocked (3%) and shocked gneisses (73%) observed by Cockell *et al.* (2002). The most probable reason for this difference lies in the increased porosity of the shocked samples, a factor of 25 higher than that found in the unshocked samples. It is quite likely that the degree of colonization of a sample is directly related to its porosity. In this case, the increased porosity of the shocked gneiss samples would provide would-be colonizers with a ready means to access the interior of the rock and greatly facilitate colonization. This suggests that, if heterotrophic endolithic communities exist at the interior of rocks, they would be significantly more widespread among samples of shocked gneiss than among samples of unshocked gneiss.

Sample Collection

Samples of shocked gneiss were aseptically collected by Dr. Charles Cockell (British Antarctic Survey) from the exposed surface of the Haughton impact structure during the summer of 2001. Samples of unshocked and shocked gneiss and breccia were retrieved from two locations within the impact structure: an isolated hill of impact melt rocks at 75°24.53′N, 89°49.76′W; and an escarpment of melt rocks (the 'Bruno Escarpment') at 75°23.9′N, 89°31.6′W. At the first site, only samples that underwent a shock greater than roughly 10 GPa were found, whereas at the latter location samples were found shocked to varying degrees, including less than 10 GPa. In this study, the term 'unshocked' gneiss corresponds to those samples exposed to a maximum shock pressure less than 5 GPa, and 'shocked' gneiss to those samples exposed to between 25 – 45 GPa. All samples collected were between 7 cm and 15 cm in diameter. Following collection, samples were placed in sterile bags and frozen with ice at approximately 0°C and returned to the British Antarctic Survey (BAS) where they were stored at -20°C.

Physical Characterization of Samples

Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) Analysis

Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) analysis was applied to samples of unshocked and shocked gneiss, and breccia to determine their bulk

(volume) mineralogical composition. Three samples of unshocked gneiss, three samples of breccia, and five samples of shocked gneiss were analyzed in duplicate. For analysis, the samples were ground into a fine powder. Each sample was prepared by a potassium hydroxide fusion to determine major element concentrations and an HF digest to determine trace element concentrations, prior to being run on the ICP-AES machine.

The following methodology was used for the preparation of samples by potassium hydroxide fusion for obtaining major element concentrations. First, 0.2 g ± 0.0005 g of each powdered sample were placed into a nickel crucible, to which ≈ 2 g of potassium hydroxide pellets were added. The crucibles were covered with a clean lid and then heated until the bottom of the crucibles began to glow red. They were then swirled to ensure the contents were thoroughly mixed and heated for one additional minute, before the crucibles were allowed to cool to room temperatures. After having cooled for 10 minutes, 70 ml of distilled water were added to each crucible. The crucibles were placed on a warm hot plate (setting 2/3) for ten minutes. Following this, the lids were rinsed into the crucibles with a small amount of water and removed, and a clean polytetrafluoroethylene (PTFE) rod was placed into each crucible to stir the contents. The crucibles remained on the warm hot plate for a further 10 minutes, after which they were removed from the hot plate, stirred once more, and allowed to cool to room temperature. Subsequently, 10 ml HNO_3 and then 20 ml of a Gallium internal standard (prepared by placing 203.8 g of concentrated Gallium into a 1L flask and adding distilled water to make 1 kg of the standard) were added to each crucible under continual stirring with the PTFE rod. Finally, 5 ml of prepared sample from each crucible were placed into labeled test tubes, to which 5 ml of distilled water was added. The labeled test tubes were stored overnight at room temperature and the samples were run on the ICP-AES machine the following morning.

The following methodology was used for the preparation of samples by HF fusion for obtaining trace element concentrations. First, 0.2 g ± 0.0010 g of each powdered sample were placed into 25 ml PTFE crucibles. Next, 6 ml of a 1:2 mixture of $HClO_4$ and HF acid were added to each crucible, after which they were placed on a hotplate (setting 3.5 – 4) in a fume cupboard and allowed to evaporate (~ 3 – 4 hours). The crucibles were then allowed to cool to room temperatures. Subsequently, 2 ml of concentrated HCl were added to each crucible and then distilled water was added to each crucible until they were filled approximately ¾ full, after which they were placed on a warm hotplate (setting 2/3) for 15 – 20 minutes. Finally, the

crucibles were allowed to cool to room temperature and 20 ml of fluid from each crucible were placed into labeled test tubes, which were stored overnight at room temperature and run on the ICP-AES machine the following morning.

Biological Characterization of Samples

Isolation of Bacteria

Samples of shocked gneiss and breccia were broken open inside sterile bags using a rock hammer. Fragments from the interior of the sample were carefully removed under sterile conditions in a laminar flow hood. The interiors of these fragments were scraped using a sterile blade into a Petri dish containing 4% tryptone-soy agar (TSA) (Difco Ltd., UK). After two days incubation at 15°C, colonies had begun to grow around the pieces of rock fragments (Figure 4). The colonies were subsequently streaked onto TSA plates until single isolates had been obtained from the original growth around the rock fragments. These colonies were maintained on TSA sloped agar tubes at 15°C.

16S rDNA Sequencing of Isolates

Prior to the 1980s the development of a taxonomy for the prokaryotes was hindered by the inability to group organisms on criteria that reflected their evolutionary origins (Franzmann, 1996). Species were grouped by shared biochemical, physiological, or morphological criteria, of unknown evolutionary significance, which were often inadequate in defining natural groupings (Woese, 1987). Zuckerkandl & Pauling (1965) suggested that evolutionary relationships between organisms could be measured by the sequence divergence in information-containing molecules. Due to its conserved nature, ribosomal RNA (rRNA) was recognized as a good source from which to construct evolutionary phylogenies (Woese & Gutell, 1989; Woese, 1987; Woese *et al.*, 1975; De Ley, 1974). The use of rRNA sequencing led to the recognition of the three superkingdoms of life and introduced the era of modern phylogenetics (Woese & Fox, 1977).

With the development of reverse transcriptase sequencing of small subunit rRNA (Lane *et al.*, 1985), polymerase chain reaction (PCR) amplification (Saiki *et al.*, 1988), sequencing of rDNA genes (Edwards *et al.*, 1989), and the establishment of nucleic acid databases (Larsen *et al.*, 1993), a revised taxonomy, based on genetic evolution, has been established. This has led to a dramatically enhanced understanding of the evolutionary relationships between organisms.

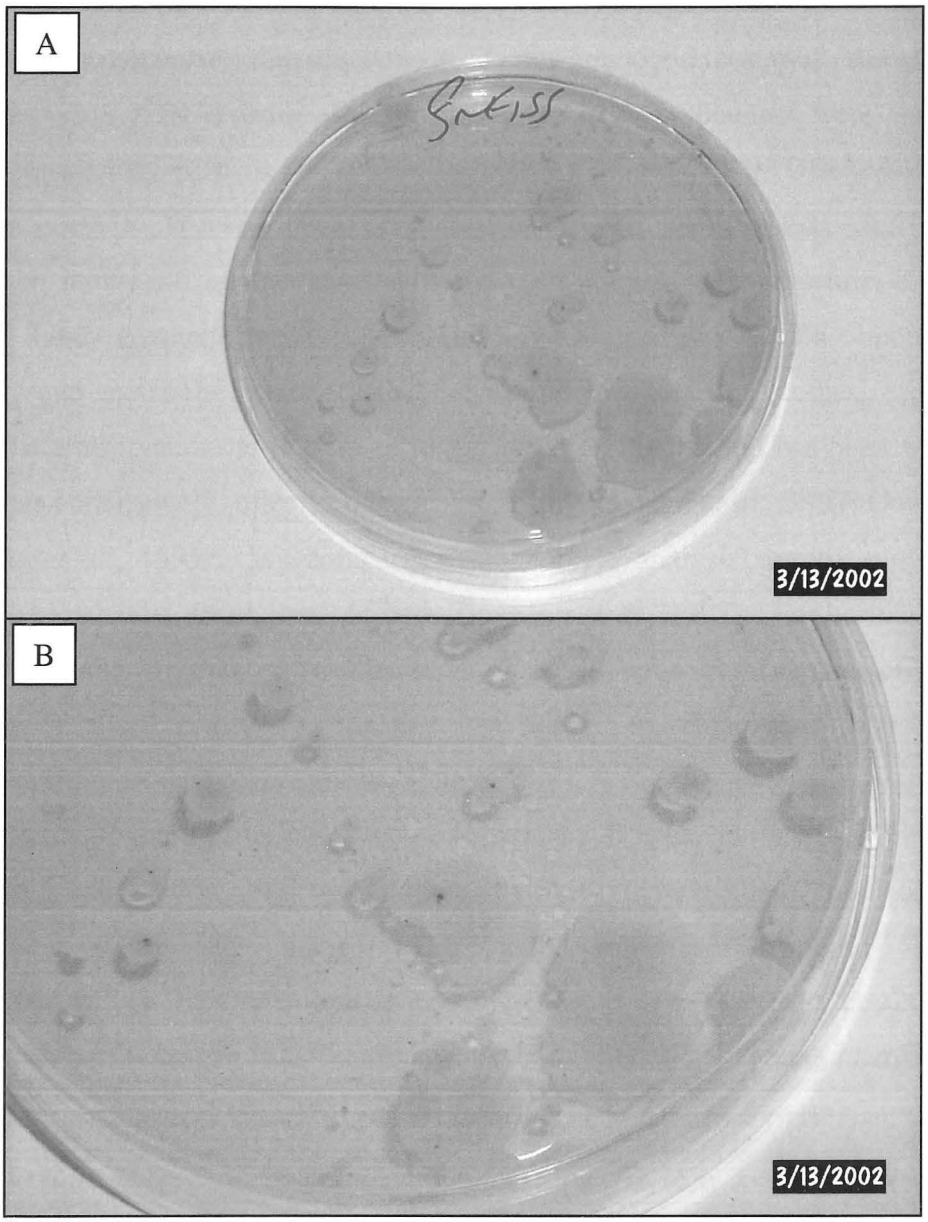

Figure 4: Isolation of bacteria from interior fragments of shocked gneiss. (a) Bacteria growing off fragments of shocked gneiss in a Petri dish. (b) Close-up showing bacterial colonies growing only around fragments of the shocked gneiss.

However, it has made it difficult to put much of the work conducted prior to the mid-1980s into context, because many of the genera that had earlier been reported from Antarctic habitats have since been redefined (Franzmann, 1996). Given the atypical nature of most Antarctic isolates, it is probable that most isolates previously assigned to genera were, in fact, novel species and the redefined taxonomy has obscured the nature of many of these organisms.

In this study, polymerase chain reaction (PCR) was employed to amplify the genes encoding the 16S rRNA (16S rDNA) from each isolate. The PCR amplicants were then sequenced subsequently to identify each isolate. This technique (Palmer, 2000; Olsen & Woese, 1993; Woese *et al.*, 1990; Woese, 1987) enables the direct identification and phylogenetic classification of individual microorganisms based upon their genetic structure, eliminating the uncertainties (and frequent errors) associated with analyses based upon phenotypic characteristics and metabolic processes that have hindered previous studies, especially those dealing with heterotrophic bacteria (Hirsch *et al.*, 1988). This method has been widely used to identify isolates conclusively, often to the species level (Bowman *et al.*, 1997; Gosink & Staley, 1995; Nichols *et al.*, 1995). By comparison, phenotypic analysis usually only succeeds in identifying isolates to the genus level, at best (Bowman *et al.*, 1997). Furthermore, 16S rDNA sequencing eliminates the risk of misidentification based upon observations of physiological and/or metabolic characteristics, a risk that can not be quantified and whose effects can propagate from study to study (Franzmann, 1996).

Amplification of the 16S rDNA was performed by Dr. David Pearce (British Antarctic Survey) on DNA extracted from bacterial cultures by freeze-thaw cycling. Then 16S rDNA gene fragments were amplified using PCR with universal bacterial primers 8F (AGAGTTTGATCCTGGCTCAG) and 1500R (AGAAAGGAGGTGATCCAGCC). Primers were synthesized by Invitrogen Life Technologies (Paisley, UK). Cell lysate (2 µl) was added to 1 µl (20 pmol) of each primer and 45 µl of ReddyMix PCR Master Mix (ABgene, Surrey, UK) in a 0.5 ml Eppendorf tube. The reaction mixture was placed in a Techne temperature cycler (Techne, Cambridge, UK) and amplification was conducted in the following manner: an initial denaturation step of 94 °C for 5 minutes, then 30 cycles of denaturation at 94 °C for 1 minute, annealing temperature 55 °C for 1 minute, and primer extension for 1 minute at 72 °C. A final annealing step of 5 minutes at 72 °C was added at the end of the reaction. All DNA extraction procedures and manipulations were carried out in a laminar flow hood to minimize aerial

contamination and all plasticware and equipment were exposed to 254 nm UV radiation for 15 minutes in a UV crosslinker (UVTech, Cambridge, England). All PCR products (10 µl volumes) were analyzed by electrophoresis in 2 % (wt/vol) agarose gels before further analysis was performed. The optimal template concentration was determined by using serial dilutions and subsequent amplification of 1 µl as template DNA in a PCR.

Aliquots of amplified rDNA were subjected to separate restriction enzyme digests for 3 hours at 37°C with the tetrameric restriction enzymes Bsu R1 and Csp 6I (Helena Biosciences), 5.0 µl of purified PCR product was placed into a 0.2 ml Eppendorf tube with l.0 µl 10x enzyme buffer, 3.6 µl distilled water and 0.4 µl of the respective restriction enzyme. The digestion products underwent electrophoresis in a 3% high-resolution agarose gel (Appligene) for 2 hours at 100V. A 1kb-plus ladder (Helena Biosciences) was included in the gel to allow for subsequent normalization. Gel images were analyzed using GelcomparII software (Applied Maths) and clones producing identical patterns with both enzymes were grouped into discrete operational taxonomic units (OTU's). At least one clone that was representative of each OTU was sequenced with both the M13F (CGCCAGGGTTTTCCCAGTCACGAC) and M13R (GAGCGGATAACAATTTCACACAGG) primers using the Big Dye terminator kit v.2 (Applied Biosystems). Sequence reactions were carried out at the University of Cambridge, Department of Biochemistry, and were run on an ABI Prism 3700 DNA analyzer (Applied Biosystems). Clone sequences were compared with the Genbank nucleotide data library using GAPPED-BLAST searches (at http://www.ncbi.nlm.nih.gov/blast/blast.cgi) (Altschul *et al.*, 1997) to determine their closest phylogenetic neighbors.

Microscopic/Microanalytic Characterization of Samples

Microscopic and microanalytic techniques were used to examine micron-scale surface morphology and composition and observe their spatial variation. Scanning electron microscopy (SEM) was applied to samples of shocked and unshocked gneiss and breccia. Application of SEM yields topographical images of the sample surface at micron-scale (Koval, 2000). SEM has been used extensively to image endolithic microorganisms in their environment and to provide information about the size and shape of individual pore spaces, their interconnectivity, and the degree of microbial colonization throughout the interior of these samples (Cockell *et al.*, 2002; Cockell & Lee, 2000; Nienow & Friedmann, 1993; Friedmann, 1982). However, this technique

only provides an external morphological description of putative biological organisms and is incapable of distinguishing between abiotic structures of similar morphologies. In order to identify bacteria within our samples conclusively, energy dispersive X-ray (EDX) microanalysis was also applied to samples of shocked and unshocked gneiss. EDX was not applied to breccia samples because the micron-scale topographic variation of these samples was too pronounced for accurate results. EDX identifies the surface composition of the sample by recording the spectrum of X-rays emitted while the sample is illuminated by an electron beam (Koval, 2000). This combination of SEM and EDX has been used to search for and identify microfossils because it synthesizes morphological (SEM) and chemical (EDX) information (Wierzchos & Ascaso, 2002; Barker *et al.*, 1997). The need for both morphological and chemical data, when determining the authenticity of microfossils and/or microorganisms, has been highlighted after recent suggestions that microfossil-like structures identified solely by SEM are in fact abiotic biomorphs, inorganic formations with shapes that resemble living organisms (e.g., Steele *et al.*, 1998).

Scanning Electron Microscopy (SEM) Analysis

Scanning Electron Microscopy (SEM) was applied to the interior of samples of shocked and unshocked gneiss, and breccia. The purpose of this analysis is two-fold: first, to identify the micro-scale effects of the impact-shock process on the samples, particularly related to the porosity of the samples; and secondly, to locate and identify microorganisms living within cracks and cavities of the samples. Interior fragments (with dimensions approximately 0.5 cm x 0.5cm x 0.5 cm) of samples were mounted on SEM stubs with epoxy adhesive. These were then coated with gold for secondary electron SEM imaging using a Cambridge Instruments (LEO) Stereoscan S360 Scanning Electron Microscope. Images were captured using a LEO Instruments PCIT image capture system and stored as standard computer TIFF files.

Energy Dispersive X-ray (EDX) Microanalysis

Energy Dispersive X-ray (EDX) microanalysis was applied to samples of both unshocked and shocked gneiss to determine their average surface composition and its micro-scale variations. Samples of unshocked and shocked gneiss were thin-sectioned, polished, and carbon coated for X-ray analysis using an Oxford Instruments EDX/INCA Energy X-ray microanalysis

system fitted with a germanium detector. Average surface composition was determined by integrating the reflected spectra over a surface area of approximately 10^4 μm^2. Micro-scale variations in surface composition were identified by conducting transects along the surface of the samples. Each transect consisted of ten points, each sampling a surface area of 1 μm^2, separated by 5 μm. Additionally, the EDX analysis was used to provide information about the surface concentration of biologically important nutrients (such as chlorine, calcium, potassium, phosphorus, etc.), which could play an important role in supporting microbial communities that live as endoliths in the interior of these samples of shocked gneiss (Egli, 2000).

Chapter 3
Results

Physical Characteristics of Samples

Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) Analysis

The ICP-AES analysis of unshocked and shocked gneiss and breccia from Haughton Impact structure identified the bulk mineral composition of the samples as well as the trace element concentrations within the samples. Samples within each of the three sample types (unshocked gneiss, shocked gneiss, and breccia) were observed to have similar compositions, both in terms of mineralogy and trace element concentrations, with the variance within each group significantly less than the difference between each group.

There is a distinct difference in mineralogy between the unshocked and shocked gneiss as well as between the gneisses and the breccia (Table 6). The primary difference observed between the average bulk chemical composition of the unshocked and shocked samples is a decrease in almost all metallic oxides, and a corresponding 10% increase in the amount of SiO_2 present within the shocked samples as compared to the unshocked samples. The following decreases were observed: Fe_2O_3 (4%); Al_2O_3 (3%); MgO (1.5%); K_2O (1.5%); Na_2O (1%); TiO_2 (0.3%); and P_2O_5 (0.14%). The decreases account for essentially all of the TiO_2 and P_2O_5, which were almost totally absent from the shocked samples. Of all the minerals identified, only the concentration of CaO in the shocked samples remained similar to its unshocked concentrations (increasing from 2.0% to 2.2%).

The breccia composition was significantly different from that of both the unshocked and shocked gneiss. The primary difference was the high percentage of MgO (9%) and CaO (23%) in the breccia samples, whereas these minerals constitute less than 3% of samples of unshocked and shocked gneiss. These elevated concentrations in the breccia agree with those measured by Metzler *et al.* (1988), and reflect the incorporation of dolomite into the breccia. Also, the breccia samples contained up to 40% volatiles, which were unable to be analyzed in this study. In comparison, volatiles composed less than 4% of the samples of unshocked and shocked gneiss.

SAMPLE	Mineral Composition (% weight)										
Shocked Gneiss	**Si2O3**	**TiO2**	**Al2O3**	**Fe2O3**	**MnO**	**MgO**	**CaO**	**Na2O**	**K2O**	**P2O5**	**Total**
sg1	84.40	0.19	8.27	1.09	0.00	0.38	0.66	0.23	2.21	0.01	97.44
sg1a	85.46	0.18	7.70	1.07	0.00	0.38	0.66	0.23	2.14	0.01	97.83
sg2	77.95	0.07	10.03	0.60	0.00	0.26	2.35	1.81	4.20	0.00	97.27
sg2a	77.67	0.07	10.08	0.60	0.00	0.25	2.31	1.81	4.21	0.01	97.01
sg3	73.90	0.14	11.85	0.81	0.01	0.23	2.19	1.85	5.63	0.01	96.62
sg3a	73.87	0.14	11.76	0.82	0.01	0.23	2.18	1.85	5.64	0.01	96.51
sg4	73.95	0.13	11.01	0.53	0.00	0.14	2.58	1.63	5.49	0.01	95.47
sg4a	75.95	0.14	11.41	0.57	0.00	0.15	2.74	1.71	5.72	0.01	98.40
sg5	75.88	0.14	10.83	0.49	0.00	0.17	3.07	1.67	5.30	0.01	97.56
sg5a	75.89	0.14	10.81	0.48	0.00	0.16	3.04	1.65	5.22	0.01	97.40
Average	**77.49**	**0.13**	**10.38**	**0.71**	**0.00**	**0.24**	**2.18**	**1.44**	**4.58**	**0.01**	**97.15**
Unshocked Gneiss	**Si2O3**	**TiO2**	**Al2O3**	**Fe2O3**	**MnO**	**MgO**	**CaO**	**Na2O**	**K2O**	**P2O5**	**Total**
ug1	71.31	0.54	13.20	3.72	0.05	1.02	2.05	2.39	5.23	0.13	99.64
ug1a	71.37	0.55	13.11	3.74	0.05	1.03	2.07	2.37	5.24	0.13	99.66
ug2	67.52	0.65	14.71	5.36	0.03	2.45	2.57	3.03	3.41	0.24	99.97
ug2a	67.12	0.66	14.99	5.46	0.04	2.51	2.59	3.06	3.50	0.24	100.17
ug3	64.89	0.08	14.92	1.52	0.04	1.16	1.56	1.66	9.30	0.09	95.22
ug3a	65.61	0.08	15.46	1.54	0.04	1.19	1.61	1.74	9.67	0.09	97.03
Average	**67.97**	**0.43**	**14.40**	**3.56**	**0.04**	**1.56**	**2.08**	**2.38**	**6.06**	**0.15**	**98.62**
Breccia	**Si2O3**	**TiO2**	**Al2O3**	**Fe2O3**	**MnO**	**MgO**	**CaO**	**Na2O**	**K2O**	**P2O5**	**Total**
b1	21.67	0.19	4.34	1.64	0.03	8.50	24.26	0.10	1.17	0.03	61.93
b1a	21.70	0.20	4.26	1.61	0.03	8.37	23.29	0.10	1.16	0.04	60.76
b2	22.90	0.23	4.77	1.67	0.03	8.49	23.73	0.12	1.53	0.04	63.51
b2a	22.96	0.23	4.84	1.72	0.03	8.58	24.07	0.12	1.56	0.04	64.15
b3	24.82	0.21	4.83	1.85	0.03	9.68	21.89	0.14	1.33	0.04	64.82
b3a	25.87	0.21	4.79	1.79	0.03	9.53	21.63	0.14	1.31	0.04	65.34
Average	**23.32**	**0.21**	**4.64**	**1.71**	**0.03**	**8.86**	**23.15**	**0.12**	**1.34**	**0.04**	**63.42**

Table 6: Mineralogy of Unshocked Gneiss, Shocked Gneiss, and Breccia.

Differences in the concentrations of trace elements within the samples were also observed (Table 7). All samples were observed to contain nutrients (particularly Zn, V, Cr, and Ni), which are necessary to sustain bacterial communities (Egli, 2000). In all cases, concentrations of each trace element in the unshocked gneiss were significantly higher than those of the shocked gneiss, and in the majority of cases (except Cr, Cu, Li, and Ni) concentrations were higher in unshocked gneiss than in the breccia. Concentrations were generally similar between shocked gneiss and breccia samples.

Sample	Trace Element Concentrations (ppm)																			
Shocked Gneiss	**Ba**	**Co**	**Cr**	**Cu**	**Li**	**Ni**	**Sc**	**Sr**	**V**	**Y**	**Zn**	**Zr**	**La**	**Ce**	**Nd**	**Sm**	**Eu**	**Dy**	**Yb**	**Pb**	**Total**
sg1	583	0	14	10	21	8	2	52	12	4	17	96	12	16	19	-0.2	0.3	0.4	0.2	9	875.8
sg1a	570	0	14	9	19	8	2	51	12	4	20	91	11	18	16	0.3	0.3	0.4	0.2	20	866.2
sg2	1144	-2	1	7	11	4	1	116	-1	3	9	179	23	42	18	0.6	0.6	0.4	0.2	15	1572.3
sg2a	1162	-2	1	8	12	4	1	118	-1	4	9	190	24	43	18	-0.4	0.6	0.5	0.2	17	1608.8
sg3	908	-2	2	7	12	4	5	116	0	44	11	175	48	97	46	7.4	0.5	5.9	2.8	14	1503.3
sg3a	882	-2	2	6	11	5	5	112	-1	39	11	171	52	100	50	7.5	0.6	5.7	2.7	14	1473.6
sg4	659	-3	2	5	12	5	3	164	-1	17	7	206	76	139	66	7.3	0.6	3.3	0.6	16	1384.8
sg4a	692	-2	2	5	11	4	4	170	-1	18	7	138	82	150	64	7.8	0.6	3.6	0.6	13	1369.9
sg5	689	-2	1	5	11	4	5	208	-1	30	6	170	113	232	110	14.8	0.8	6.6	1.5	16	1619.8
sg5a	706	-2	1	5	11	4	5	213	-1	27	6	160	104	208	96	13.2	0.7	6.1	1.4	12	1576.3
Average	800	-2	4	7	13	5	3	132	2	19	10	158	55	105	50	5.8	0.5	3.3	1.0	15	1385.1
Unshocked Gneiss	**Ba**	**Co**	**Cr**	**Cu**	**Li**	**Ni**	**Sc**	**Sr**	**V**	**Y**	**Zn**	**Zr**	**La**	**Ce**	**Nd**	**Sm**	**Eu**	**Dy**	**Yb**	**Pb**	**Total**
ug1	1641	4	5	14	21	8	9	412	35	36	53	374	86	171	93	14.2	1.7	6.3	2.3	18	3004.4
ug1a	1636	4	4	14	21	6	9	414	36	36	53	349	85	172	92	14.3	1.7	6.3	2.3	17	2972.9
ug2	656	10	18	15	32	21	6	301	51	18	54	315	92	175	83	10.3	1.1	3.3	0.9	12	1874.4
ug2a	666	10	18	14	34	22	7	309	52	18	56	263	99	187	84	10.8	1.2	3.3	0.8	10	1865.4
ug3	5579	-2	5	4	18	5	6	1474	14	26	40	16	263	619	337	44.7	11.1	6.7	0.9	25	8492.5
ug3a	5738	-2	5	4	18	5	6	1536	14	27	41	26	275	652	358	45.9	11.3	6.8	0.9	26	8793.3
Average	2653	4	9	11	24	11	7	741	34	27	50	224	150	329	175	23.4	4.7	5.5	1.4	18	4500.5
Breccia	**Ba**	**Co**	**Cr**	**Cu**	**Li**	**Ni**	**Sc**	**Sr**	**V**	**Y**	**Zn**	**Zr**	**La**	**Ce**	**Nd**	**Sm**	**Eu**	**Dy**	**Yb**	**Pb**	**Total**
b1	259	2	18	16	42	12	4	220	22	11	25	80	25	44	31	1.0	0.5	1.1	0.8	11	825.3
b1a	259	2	19	15	41	13	4	221	22	11	26	85	23	40	36	1.4	0.5	1.1	0.8	17	838.5
b2	265	2	18	16	42	13	4	195	26	11	26	91	21	35	36	1.0	0.4	1.2	0.8	14	818.1
b2a	273	2	19	16	43	13	4	201	28	11	26	93	25	38	38	0.3	0.5	1.0	0.8	17	848.9
b3	304	2	20	20	55	13	4	195	25	12	29	89	25	46	39	1.4	0.5	1.4	0.8	12	895.0
b3a	305	2	19	14	54	13	4	195	24	12	28	103	26	49	38	2.0	0.5	1.6	0.9	12	902.7
Average	278	2	19	16	46	13	4	205	25	11	27	90	24	42	36	1.2	0.5	1.2	0.8	14	854.7

Table 7: Trace Element Concentrations of Unshocked Gneiss, Shocked Gneiss, and Breccia.

ICP-AES analysis of our samples enabled us to place them into the context of the different crystalline rock types at Haughton, as identified by Metzler *et al.* (1988), by comparing their respective chemical compositions. The samples of shocked gneiss used in this study have a chemical composition that is closest to the aplitic alkali feldspar gneisses within the group of alkali feldspar-rich gneisses, followed by the felsic sillimanite gneisses within the group of sillimanite- and garnet-bearing gneisses, whereas our samples of unshocked gneisses most closely resemble the biotite-bearing gneisses and hornblende gneisses within the group of biotite-bearing gneisses from Metzler *et al.* (1988).

Biological Characterization of Samples

Isolated Heterotrophic Bacteria

A total of 27 bacteria were isolated from the interiors of samples of shocked rocks (14 from shocked gneiss; 13 from breccia). A representative sample of the appearance of the isolates is shown in Figure 5. The majority of the isolates had little pigmentation (color ranged from clear to light yellow), although some (particularly isolate G50) contained significant concentrations of carotenoids and other pigments.

The bacterial isolates from samples of shocked gneiss and breccia, were selected, along with three isolates from Antarctic soil as a comparison, to undergo sequencing of their 16S rDNA genes. This sequencing process was successful for 13 out of 14 isolates from the shocked gneiss samples, for all 13 isolates from the breccia samples, and for all three of the Antarctic soil isolates. Isolate G20 from the gneiss sample did not amplify properly during the PCR stage, preventing a proper sequence from being obtained. This isolate was observed to possess a thick waxy coating when observed under an optical microscope. This waxy coating is the likely cause of the failure of the amplification process because excess polysaccharides can inhibit the PCR reaction (Demeke & Adams, 1992; Do & Adams, 1991).

Of the 13 shocked gneiss isolates successfully sequenced, 9 had sequences significantly similar to those found in the GenBank database and have been identified to the species level (see Table 3). Isolates with sequences over 200 base pairs can be matched with statistical significance (Altschul *et al.*, 1997). An isolate with a sequence that matches one in the database to 97% or higher can be considered to be the same species as the database species, whereas sequences matching about 70% can be considered to be in the same genus (Altschul *et al.*, 1997). The sequences for the remaining 4 shocked gneiss isolates (G11, G0, G13, G14) showed no significant similarity to any sequence within the database (having matches that at best were less than 20 base pairs in length). Of the 13 breccia isolates that were sequenced, 11 were found to match significantly sequences from the GenBank database. The two other breccia isolates (B8 and B11) had sequences that did not match any of the database sequences to a significant level. The three Antarctic soil isolates that were sequenced all matched sequences from the GenBank database. The percentage match between our isolates and the sequence in the database was determined by examining the matching base pairs within the total sequence.

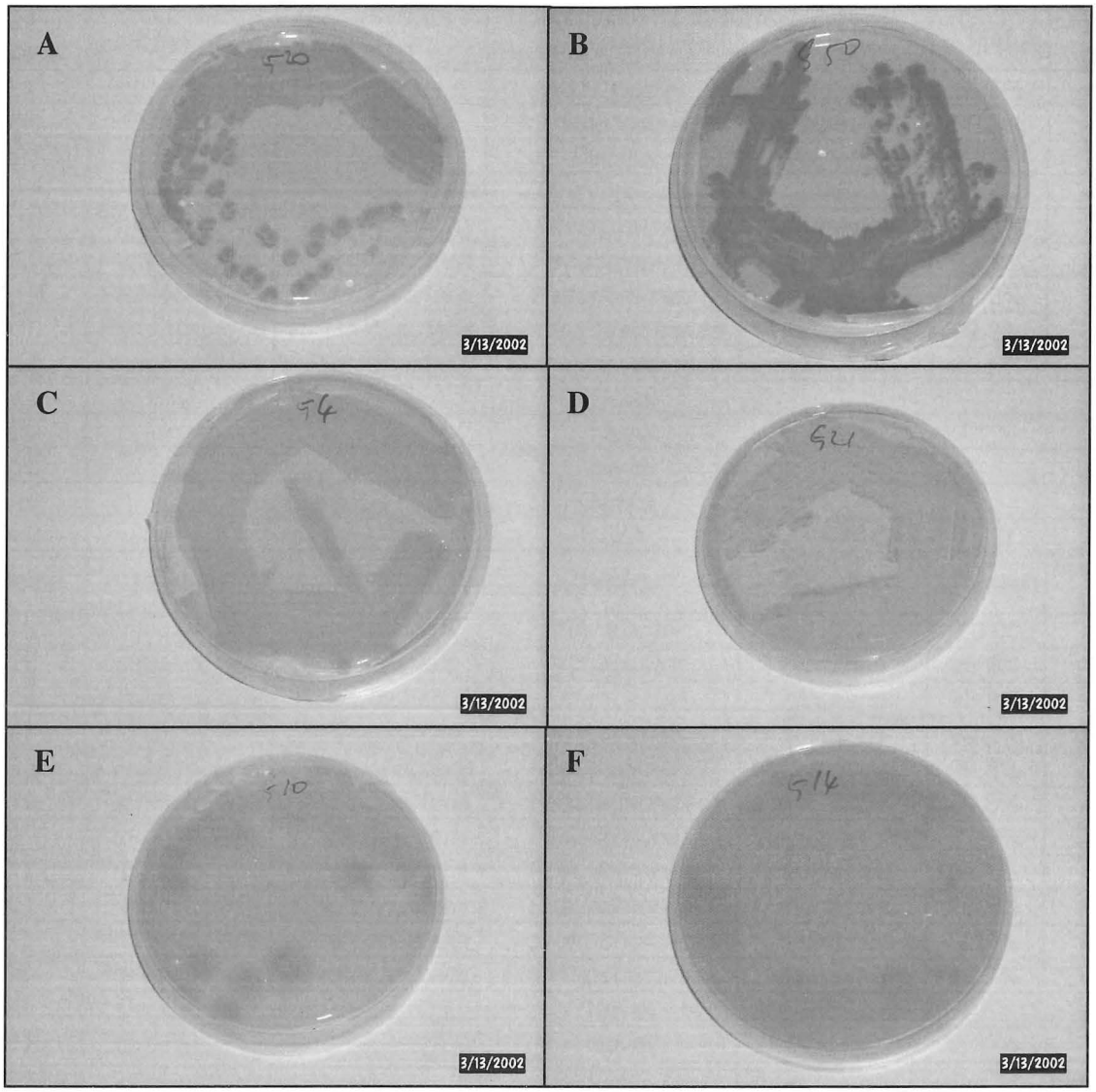

Figure 5: Bacterial isolates obtained from the interior of samples of shocked gneiss. (a) Coccoid bacterial isolate G20. When observed under an optical microscopy, the bacteria appear to have a waxy outer layer. (b) Coccoid bacterial isolate G50. Note the pigmentation caused by caratenoid production. (c) Coccoid bacterial isolate G4. (d) Rod bacterial isolate G21. (e) Rod bacterial isolate G10. (f) Rod bacterial isolate G14. Tables 8 and 9 identify and describe these, and the remaining, isolates.

The identification of our isolates with known species in the GenBank database provides us with several means to characterize the bacteria, including phylogeny, habitat, and metabolism. Phylogenetically, the isolated bacteria from the shocked gneiss and breccia were similar to each other, and to those isolated from Antarctic soil (See Table 8).

Shocked Gneiss	Phylum	Class	Genus	Species	% Match	Matches (bp)
G4	*Actinobacteria*	*Actinobacteria*	*Arthrobacter*	*nicotiana*	98.5%	460
G50	*Firmicutes*	*Bacilli*	*Planococcus*	*citreus*	99.3%	456
G21	*Firmicutes*	*Bacilli*	*Bacillus*	*psychrophilus*	97.2%	447
G23	*Proteobacteria*	*β-Proteobacteria*	*Janthinobacter*	*lividum*	99.4%	464
G10	*Proteobacteria*	*γ-Proteobacteria*	*Pseudomonas*	*rhodesiae*	99.8%	473
G22	*Proteobacteria*	*γ-Proteobacteria*	*Pseudomonas*	*borealis*	98.9%	465
G28	*Proteobacteria*	*γ-Proteobacteria*	*Pseudomonas*	*grimontii*	99.6%	781
G16	*Proteobacteria*	*γ-Proteobacteria*	*Stenotrophomonas*	*maltophilia*	99.3%	761
G15	*Proteobacteria*	*γ-Proteobacteria*	*Stenotrophomonas*	*maltophilia*	99.6%	465
G11	Novel					
G0	Novel					
G13	Novel					
G14	Novel					
G20	N/A					
Breccia	**Phylum**	**Class**	**Genus**	**Species**	**% Match**	**Matches (bp)**
B10	*Actinobacteria*	*Actinobacteria*	*Arthrobacter*	*globiformis*	100.0%	474
B24	*Actinobacteria*	*Actinobacteria*	*Arthrobacter*	*nicotiana*	98.9%	470
B16	*Actinobacteria*	*Actinobacteria*	*Arthrobacter*	*nicotiana*	98.9%	470
B4	*Actinobacteria*	*Actinobacteria*	*Arthrobacter*	*sulfureus*	99.2%	730
B20	*Proteobacteria*	*α-Proteobacteria*	*Caulobacter*	*bacteriodes*	96.8%	477
B12	*Proteobacteria*	*γ-Proteobacteria*	*Pseudomonas*	*IC038*	99.8%	479
B15	*Proteobacteria*	*γ-Proteobacteria*	*Pseudomonas*	*psychrophilia*	97.1%	706
B17	*Proteobacteria*	*γ-Proteobacteria*	*Pseudomonas*	*rhodesiae*	98.1%	471
B21	*Proteobacteria*	*γ-Proteobacteria*	*Pseudomonas*	*rhodesiae*	93.3%	431
B3	*Proteobacteria*	*γ-Proteobacteria*	*Stenotrophomonas*	*maltophilia*	99.0%	489
B6	*Proteobacteria*	*γ-Proteobacteria*	*Stenotrophomonas*	*maltophilia*	96.8%	479
B8	Novel					
B11	Novel					
Antarctic Soil	**Phylum**	**Class**	**Genus**	**Species**	**% Match**	**Matches (bp)**
S13	*Actinobacteria*	*Actinobacteria*	*Arthrobacter*	*CAB1*	95.9%	772
S16	*Actinobacteria*	*Actinobacteria*	*Arthrobacter*	*S23H2*	95.8%	668
S21	*Firmicutes*	*Bacilli*	*Bacillus*	*psychrophilus*	98.0%	448

Table 8: Phylogenetic Identification of Isolates from Shocked Gneiss, Breccia, and Antarctic Soil. The nearest phylogenetic species to our isolates is shown down to the species level, with the percent match between the sequence for our isolates and that found in the GenBank database. Novel indicates that there are no significant matches between the isolate sequence and any species in the database. N/A = sample G20 did not amplify properly in PCR.

The nine bacteria isolated from the shocked gneiss were clustered into the following taxonomic groups: 6 *Proteobacteria* (5 γ-*Proteobacteria* (3 *Pseudomonas* and 2 *Stenotrophomonas*) and 1 β-*Proteobacterium* (*Janthinobacter*)); 2 *Firmicutes* (1 *Bacillus* and 1 *Planococcus*); and 1 *Actinobacterium* (*Arthrobacter*). The 11 identifiable breccia isolates were clustered into the following taxonomic groups: 7 *Proteobacteria* (6 γ-*Proteobacteria* (4 *Pseudomonas* and 2 *Stenotrophomonas*) and 1 α-Proteobacterium (*Caulobacter*)); and 4 *Actinobacteria* (*Arthrobacter*). Two *Actinobacteria* (*Arthrobacter*); and 1 *Firmicutes* (*Bacillus*) were identified from the Antarctic soil isolates.

The bacteria identified from the samples of shocked gneiss and breccia are prominently soil, ice, or freshwater bacteria (Table 9). Members of the genera *Arthrobacter*, *Bacillus*, *Janthinobacter*, and *Pseudomonas* are frequently found as soil bacteria. *Arthrobacter* species are common soil bacteria and all *Arthrobacter* species identified in this study have previously been isolated from soil (Keddie *et al.*, 1984). In addition, *A. nicotiana* has been found in cave silts, glacial silts, sewage, air, and tobacco plants, and *A. sulfurous* has previously been isolated from oil brines (Keddie *et al.*, 1984). *Bacillus* species are widely distributed in nature and *B. psychrophilus* has been isolated from soil and river waters (Claus & Berkeley, 1984). *Janthinobacter lividum* is a common soil and freshwater organism, although it is most common in temperate climates (Sneath, 1984). *Pseudomonas* species are common components of soil bacterial communities, and *P. borealis*, *P. sp. IC038*, and *P. psychrophilia* have been isolated from soil environments (Palleroni, 1984). In addition to soil habitats, the bacteria identified in this study are frequently found in freshwater, marine, and/or ice environments. *Pseudomonas* species are abundant in freshwater bacterial communities and all *Pseudomonas* species isolated in this study have previously been found in freshwater environments (Palleroni, 1984). *Caulobacter bacteroides* has also been found in freshwater habitats (Abraham *et al.*, 1999). *Planococcus citreus* is a marine organism, and is often isolated from polar waters (Kocur, 1984). All *Arthrobacter* species isolated in this study have also been found in glacial ice (Keddie *et al.*, 1984). Among the identified bacteria, the only one not normally associated with soil, ice, and water was *Stenotrophomonas maltophilia*, which was found in both the shocked gneiss and the breccia, and which is customarily isolated from clinical specimens and from milk, water, and frozen foods (Palleroni, 1984)

Three bacteria, similar to those found in the shocked gneiss and breccia, were isolated from Antarctic soil samples; these consisted of two species of *Arthrobacteria* and one species of *Bacillus*. *Arthrobacter sp.* CAB1 (Morikawa *et al.*, 2002) had previously been isolated from 200-year old glacial ice in China (Christner, 2002) and from agricultural soils (Lukow, 1999). *Arthrobacter sp.* S23H2 had previously been isolated from Antarctic sea ice brine and cryoconite holes (Junge *et al.*, 1998). *Bacillus psychrophilus*, also found in our sample of shocked gniess, had previously been isolated from soil and freshwater, including from a fiord in Greenland (Suzuki & Yamasato, 1994).

Genus	Species	Shocked Gneiss	Breccia	Antarctic Soil	Other Known Habitats
Arthrobacter	*Nicotiana*	+	+	-	Soil/Glacial Ice
Arthrobacter	*Globiformis*	-	+	-	Soil/Glacial Silts
Arthrobacter	*Sulfurous*	-	+	-	Soil/Glacial Ice
Arthrobacter	*CAB1*	-	-	+	Soil/Glacial Ice
Arthrobacter	*S23H2*	-	-	+	Sea Ice Brine
Bacillus	*Psychrophilus*	+	-	+	Soil/Freshwater
Caulobacter	*Bacteriodes*	-	+	-	Freshwater
Janthinobacterium	*Lividum*	+	-	-	Soil/Freshwater
Planococcus	*Citreus*	+	-	-	Sea Ice/Glacial Ice
Pseudomonas	*Borealis*	+	-	-	Soil/Freshwater
Pseudomonas	*Grimontii*	+	-	-	Freshwater
Pseudomonas	*IC038*	-	+	-	Soil/Freshwater
Pseudomonas	*Psychrophilia*	-	+	-	Soil/Freshwater
Pseudomonas	*Rhodesiae*	+	+	-	Freshwater
Stenotrophomonas	*Maltophilia*	+	+	-	Clinical/Frozen Foods

Table 9: Known Habitats of Identified Bacteria.

The bacteria identified based on their sequence information all possess relatively similar metabolisms and physiological properties. All of the species identified are heterotrophs (chemoorganotrophs), although some species of *Pseudomonas* and *Bacillus* are known to be facultative chemolithoautotrophs (Claus & Berkeley, 1984; Palleroni, 1984). It has not been determined whether the *Pseudomonas spp.* or *Bacillus psychrophilus* identified in this study possess the ability to be facultative chemolithoautotrophs. All of the identified bacteria are motile, with the exception of the *Arthrobacter spp.*, which are nonmotile (Palleroni, 1984). The remainder are motile through a combination of polar (*Pseudomonas*, *Stenotrophomonas*, *Planococcus*), polar and subpolar/lateral (*Janthinobacter*) or peritrichous (*Bacillus*) flagella,

(Claus & Berkeley, 1984; Keddie *et al.*, 1984; Kocur, 1984; Palleroni, 1984; and Sneath, 1984). Most of the bacteria isolated are obligate aerobes, notable exceptions include select species of *Pseudomonas*, which can replace oxygen with nitrate as a terminal electron acceptor, allowing anaerobic growth (Palleroni, 1984), and species of *Bacillus*, which can replace oxygen with a variety of different terminal electron receptors, again allowing for anaerobic growth (Claus & Berkeley, 1984). Facilities were not available to determine whether the *Pseudomonas spp.* or *Bacillus psychrophilus* identified in this study can function as facultative anaerobes. All of the isolated bacteria have strictly respiratory and never fermentative metabolisms (Claus & Berkeley, 1984; Keddie *et al.*, 1984; Kocur, 1984; Palleroni, 1984; and Sneath, 1984). All of the species are nutritionally non-exacting, either requiring no growth factors (*Pseudomonas spp.*, *Janthinobacter lividum*, *Bacillus psychrophiles*, *Planococcus citreus*) or possessing basic requirements (such as methionine or cystine for *Stenotrophomonas maltophilia* (but not always, see Ikemoto et al. 1980), and biotin for *Arthrobacter spp.*) (Claus & Berkeley, 1984; Keddie *et al.*, 1984; Kocur, 1984; Palleroni, 1984; and Sneath, 1984).

The largest variation observed among the bacteria involves the temperature ranges in which they can function. Many of the bacteria identified are known to be either psychrophilic, including some of the *Pseudomonas* and *Arthrobacter* species, and *Bacillus psychrophilus*, or psychrotrophic (the remainder of the *Pseudomonas* and *Arthrobacter* species, *Janthinobacter lividum*, and *Planococcus citreus*) (Claus & Berkeley, 1984; Keddie *et al.*, 1984; Kocur, 1984; Palleroni, 1984; and Sneath, 1984). However, *Stenotrophomonas maltophilia*, with an optimum growth temperature at or above 35°C is neither and cannot normally grow at temperatures below 10°C (Palleroni, 1984). In addition to growth at 15°C, selected isolates were grown at 5°C and 20C to better understand their temperature ranges. Based upon observations of limited growth at 20°C, and/or accelerated growth at 5°C (Table 10), it appears that several isolates may be psychrophilic, particularly G50 (*Planococcus citreus*), G11 (novel), G13 (novel), B15 (*Pseudomonas psychrophilia*), and B11 (novel). It should be noted that these data represent only a preliminary characterization of the thermal regimes for each bacteria, and a more thorough study would be necessary to conclusively identify psychrophiles from psychrotrophs.

Shocked Gneiss	Genus	Species	Type	Growth at 5°C	Growth at 15°C	Growth at 20°C
G4	*Arthrobacter*	*Nicotiana*	Coccoid	+	++	++
G50	*Planococcus*	*Citreus*	Coccoid	+	++	+
G21	*Bacillus*	*Psychrophilus*	Rod	++	++	+
G23	*Janthinobacter*	*Lividum*	Rod	-	++	+
G10	*Pseudomonas*	*Rhodesiae*	Rod	-	++	-
G22	*Pseudomonas*	*Borealis*	Rod	-	++	-
G28	*Pseudomonas*	*Grimontii*	Rod	-	++	++
G16	*Stenotrophomonas*	*Maltophilia*	Rod	-	++	-
G15	*Stenotrophomonas*	*Maltophilia*	Rod	-	++	++
G11	Novel		Rod	+	++	+
G0	Novel		Coccoid	-	++	++
G13	Novel		Rod	+	++	-
G14	Novel		Rod	-	++	-
G20	N/A		Coccoid	-	++	++
Breccia						
B10	*Arthrobacter*	*Globiformis*	Coccoid	-	++	-
B24	*Arthrobacter*	*Nicotiana*	Coccoid	N/A	++	N/A
B16	*Arthrobacter*	*Nicotiana*	Coccoid	N/A	++	N/A
B4	*Arthrobacter*	*Sulfurous*	Coccoid	+	++	++
B20	*Caulobacter*	*Bacteriodes*	Rod	+	++	++
B12	*Pseudomonas*	*IC038*	Rod	-	++	++
B15	*Pseudomonas*	*Psychrophilia*	Rod	+	++	+
B17	*Pseudomonas*	*Rhodesiae*	Rod	N/A	++	N/A
B21	*Pseudomonas*	*Rhodesiae*	Rod	N/A	++	N/A
B3	*Stenotrophomonas*	*Maltophilia*	Rod	N/A	++	N/A
B6	*Stenotrophomonas*	*maltophilia*	Rod	N/A	++	N/A
B8	Novel		Rod	-	++	-
B11	Novel		Rod	+	++	-
Antarctic Soil						
S13	*Arthrobacter*	*CAB1*	Coccoid	N/A	++	N/A
S16	*Arthrobacter*	*S23H2*	Coccoid	N/A	++	N/A
S21	*Bacillus*	*psychrophilus*	Rod	N/A	++	N/A

Table 10: Summary of Physiological and Growth Characteristics for Bacterial Isolates. ++ indicates significant growth, + moderate growth, and – no growth was observed. N/A indicates these isolates were not tested at 5° or 20°. Growth tests at 5° and 20° were conducted for 96 hours.

Microscopic/Microanalytical Characterization of Samples

Scanning Electron Microscopy (SEM) Analysis

Interior fragments of unshocked and shocked gneiss and breccia were analyzed using SEM. The SEM images provide a characterization of the topography of the samples (Figure 6). It is clear from examination of the unshocked gneiss that the surface is quite homogenous and there are few, if any, pore spaces for bacteria to inhabit (Figure 6a,b). Images of shocked gneiss reveal the micro-scale effects that impact-induced shock has upon gneiss, particularly the generation of significant porosity within the samples, which could serve as bacterial habitats. (Figure 6c,d,e). Observations of the breccia show the complex topography that resulted from the impact shock to these samples (Figure 6f,g,h). Unlike the smooth surfaces present in samples of shocked gneiss, the breccia samples are a conglomeration of micron-scale features possessing a broad morphological variation. Despite the fact that these samples contain bacteria (13 bacteria were isolated from their interiors), it was not possible to visually identify microbes due to the complex topography of the breccia.

In samples of shocked gneiss, however, SEM images clearly record the presence of a variety of microorganisms (Figure 7). Both rod-shaped and coccoid microbes were observed inhabiting cavities that were created as the result of the impact (Figure 7a,b). Although rod-shaped microorganisms would usually appear singly, or in small groups, coccoid microorganisms tended to occur together in larger groups (Figure 7c,d), often covering the entire surface of cavities (Figure 7e,f). The coccoid microbes were always observed with a coating of extracellular, exopolymeric substances (EPS), which usually connected all the individuals observed together within a cavity. SEM observations regularly reveal the presence of EPS in association with microbes (e.g., Steele *et al.*, 2000).

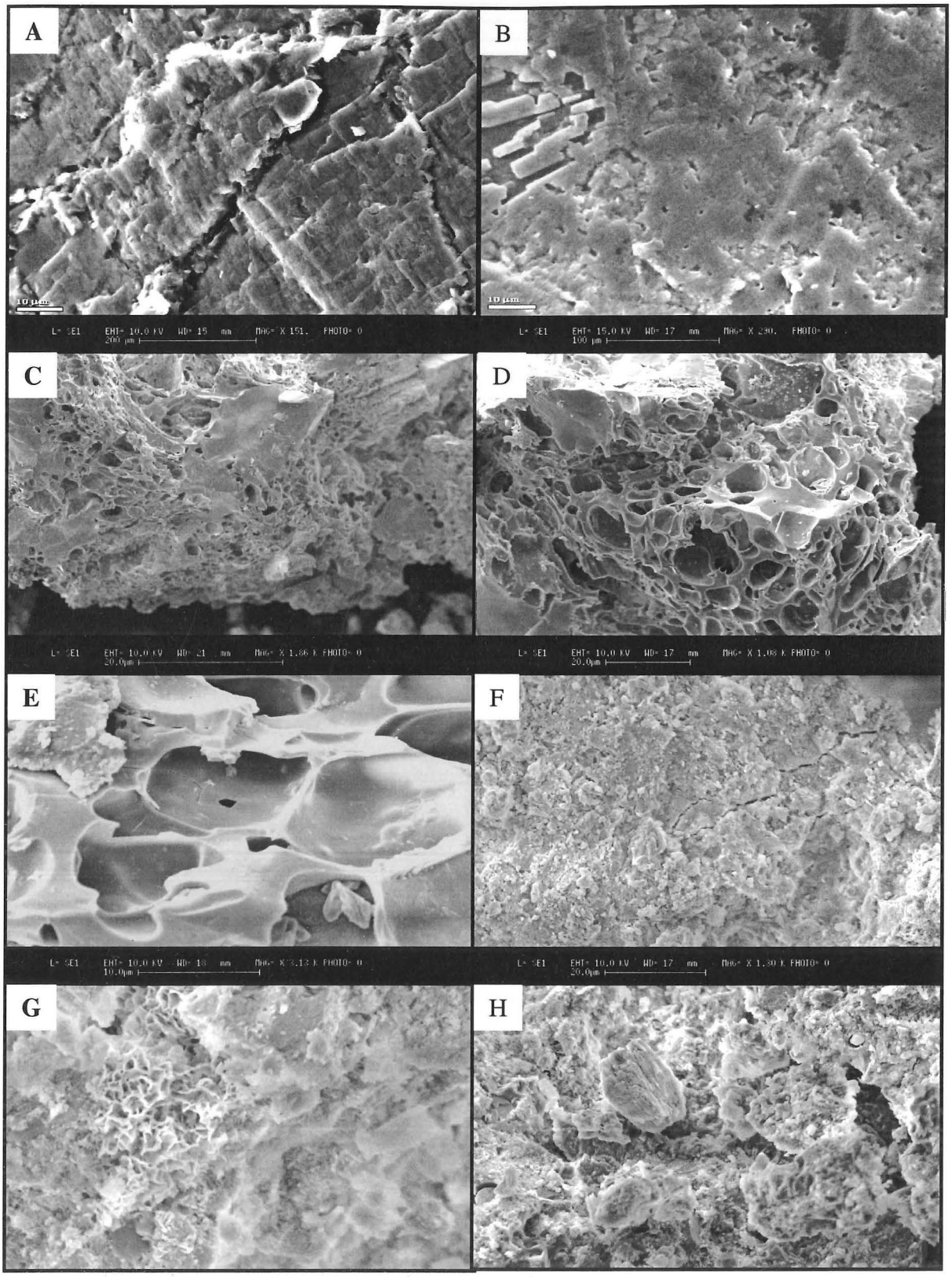

Figure 6: SEM images. (a, b) Fragment of unshocked gneiss. (c, d) Fragment of shocked gneiss. Note the variation in surface, particularly the vesicles. (e) Closer image of shocked gneiss highlighting the surface cavities. (f) Fragment of breccia. (g,h) Closer image of breccia fragment. Note the surface complexity drastically hinders visual identification of microbes.

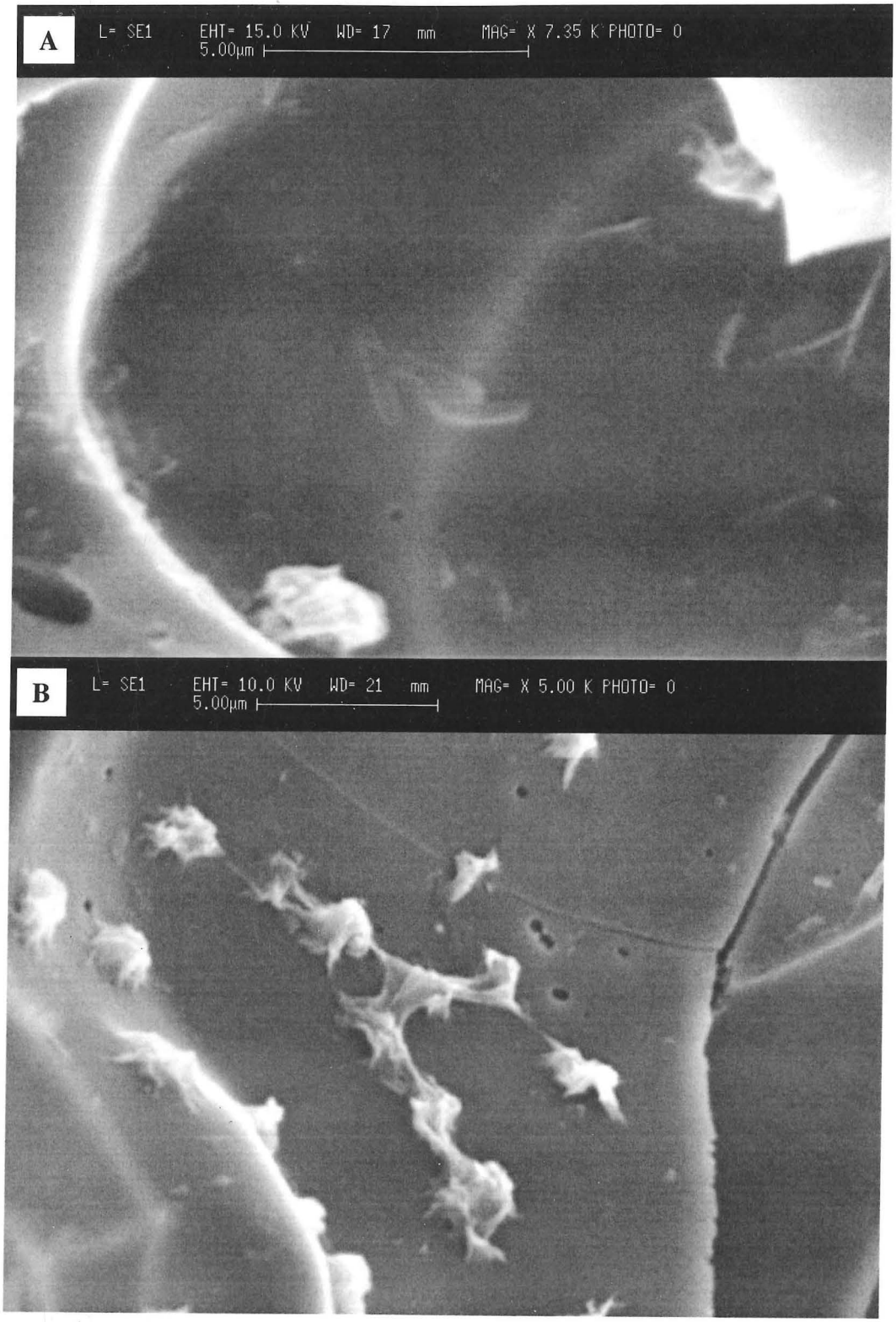

Figure 7: SEM images of bacteria on shocked gneiss. (a) Three rod-shaped bacteria in a cavity. (b) Small group of coccoid bacteria, connected by an extracellular, exopolymeric substance (EPS).

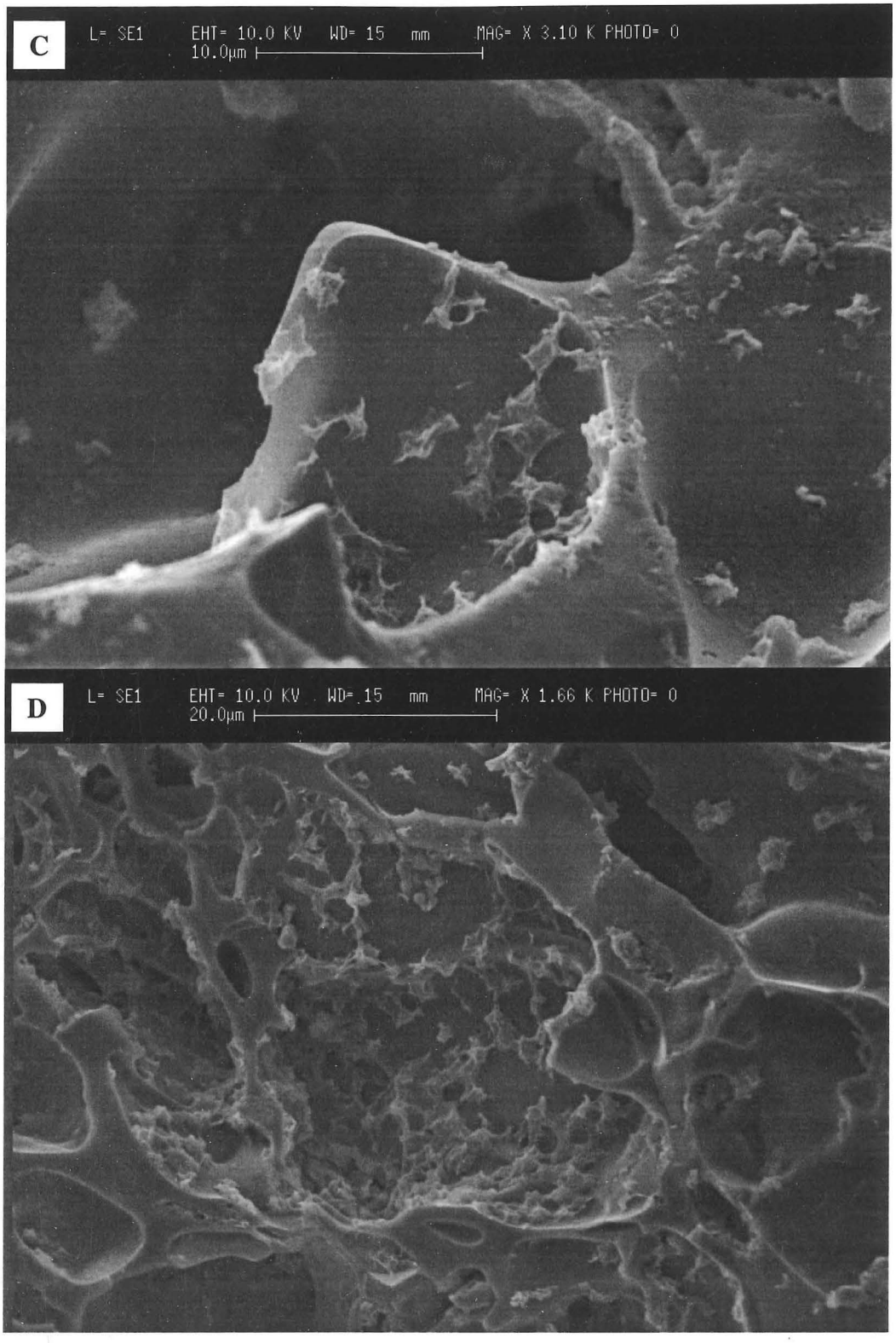

Figure 7: SEM images of bacteria on shocked gneiss. (c) Another small group of coccoid bacteria. An 'empty' sheath of EPS reveals the spherical shape of the underlying bacteria. (d) Colony of coccoid bacteria partially coating the surface of the central cavity.

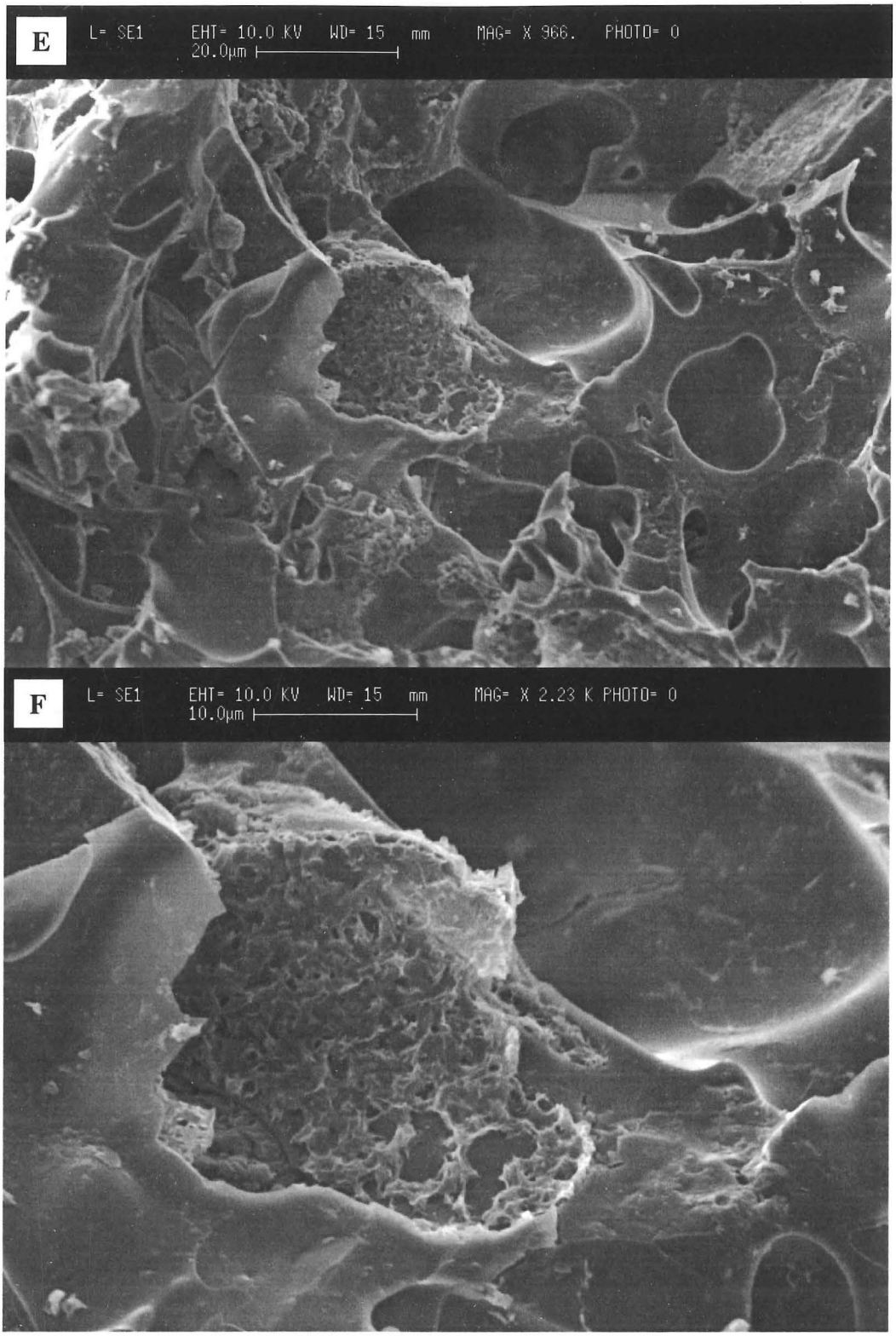

Figure 7: SEM images of bacteria on shocked gneiss. (e) Large colony of coccoid bacteria coating the entire surface of a cavity. (f) Close-up of the previous. Individual bacteria can be discerned within the biofilm.

Energy Dispersive X-ray (EDX) Analysis

Energy dispersive X-ray (EDX) analysis was applied to samples of unshocked and shocked gneiss. Repeated measurements (40) of the surfaces were conducted, with individual measurements recording composition for an area of either 10^4 μm^2 or 1 μm, to achieve an understanding of the average surface composition and its micron-scale variation. The analysis of the unshocked gneiss revealed its surface was composed solely of Si, O, Al, Fe, and trace amounts of Ti. (Figure 8a). Samples of shocked gneiss are observed to have highly heterogeneous surface composition, particularly along the edge and within the interior of impact-induced cavities. The surface of shocked gneiss *distant from surface cavities* (Figure 8b) resembled that of unshocked gneiss, although significant decreases in Al, Fe, and Ti were observed on the surface of shocked gneiss. The composition within surface cavities on shocked gneiss changed dramatically and the presence of Mg, K, Na, P, Ca, S, and Cl were detected (Figure 8c,d). In particular, the placement of these latter, biologically important elements in cavities within shocked gneiss correlates well with SEM observations of microorganisms inhabiting cavities (Figure 7). To observe the strength of a biological signal against a sterile mineral background, a sterile glass slide, representing an abiologic silicate background, and a slide with different concentrations of bacteria (isolate G20, obtained from the sample of shocked gneiss), representing microorganisms inhabiting cavities with shocked gneiss, were analyzed using EDX (Figure 8e,f,g,h). The sterile glass slide (Figure 8e) had a surface composition similar to the surface of unshocked gneiss (Figure 8a) and the surface of shocked gneiss *distant from surface cavities* (Figure 8b). The slides with isolate G20 had clearly biologic spectra, whose strength relative to the silicate background increased in proportion to the thickness of the bacteria on the slide (Figure 8f,g,h). The occurrence of chlorine in the spectra, observed even under the thinnest layer of bacteria, was the clearest and most sensitive indicator of the presence of bacteria using EDX.

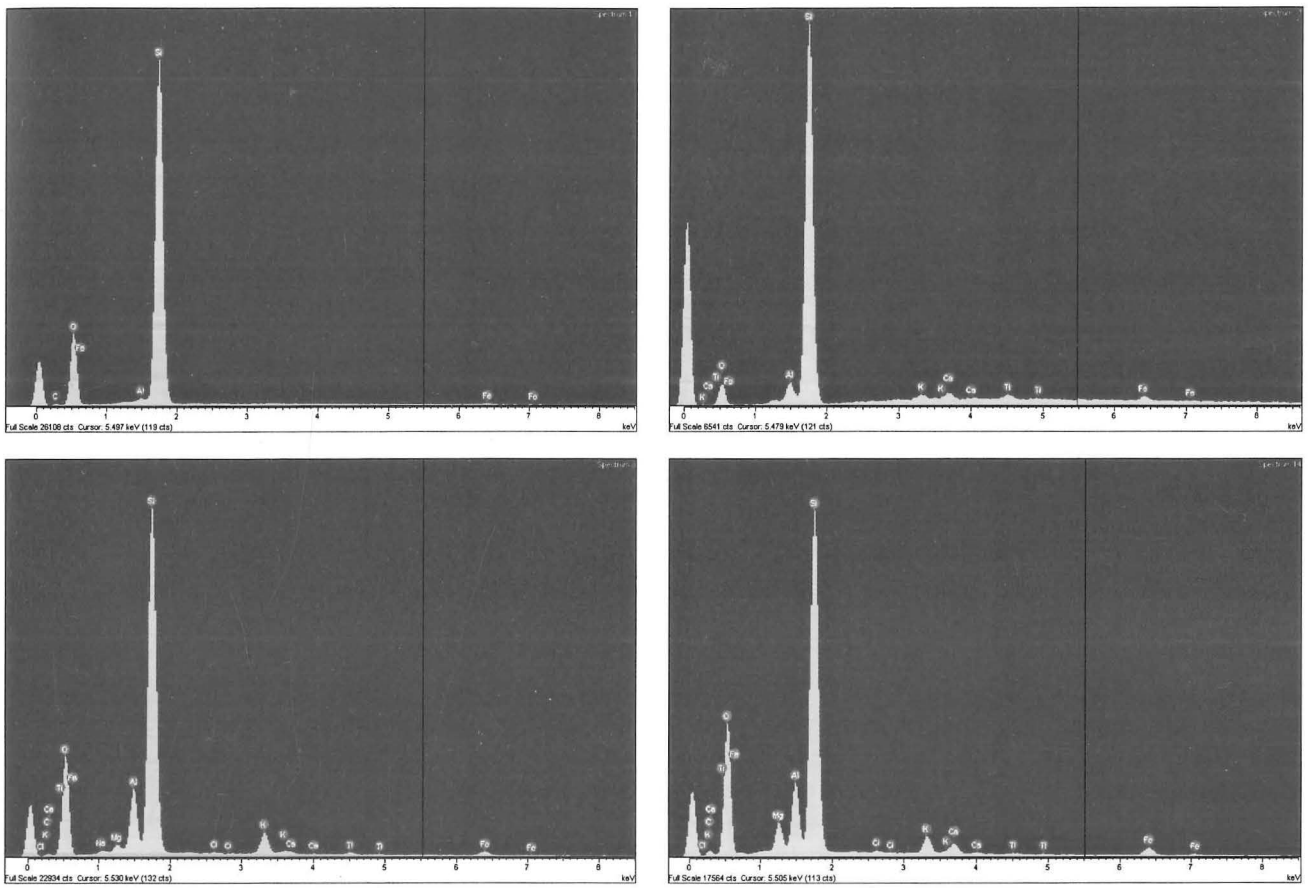

Figure 8: Energy Dispersive X-ray Spectra. (a) Surface of unshocked gneiss. (b) Surface of shocked gneiss. (c,d) Cavities present in the surface of shocked gneiss. Note the increased range of elements present at the surface in the shocked gneiss, particularly in the cavities, where a clear biological signature can be discerned. These spectra (c,d) were taken over cavities, in which SEM imaging revealed groups of coccoid bacteria (see Figure 7e)

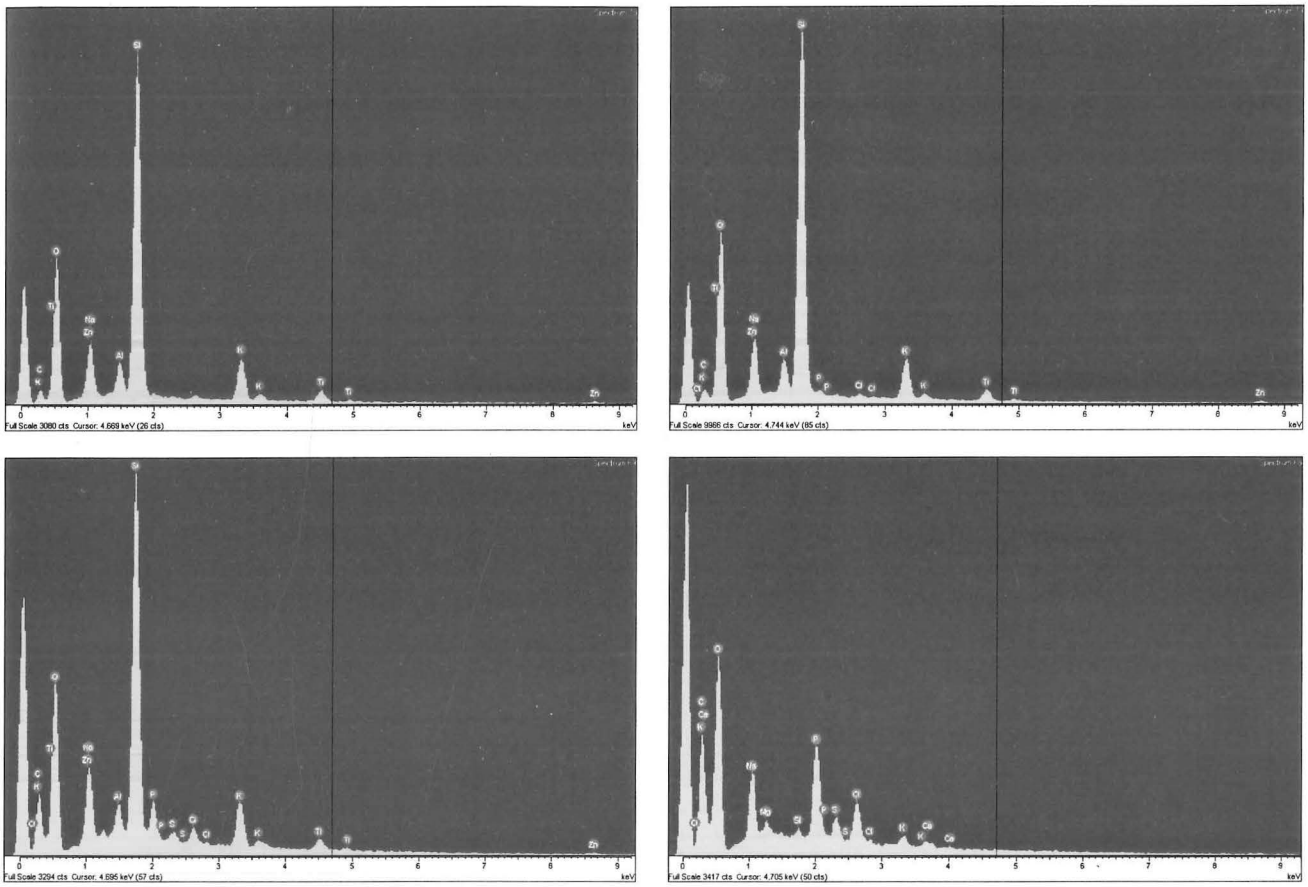

Figure 8: Energy Dispersive X-ray Spectra. (a) Sterile Glass Slide. (b) Glass slide with trace amounts (invisible to the naked eye) of bacteria (isolate G20). (c) Glass slide with single layer of bacteria (isolate G20). (d) Glass slide with thick layer of bacteria (isolate G20).

Transects conducted along the surface of unshocked (Figure 9a) and shocked (Figure 9b) gneiss reveal impact-induced changes in surface composition and illustrate the microbial habitats created by micro-scale variations in the surface of the shocked gneiss. The surface of unshocked gneiss showed relatively little variation in surface composition, even on the micron-scale. However, the composition of the shocked gneiss varied considerably over a few microns on the surface, and quite dramatically near or inside a surface cavity (right of Figure 9b).

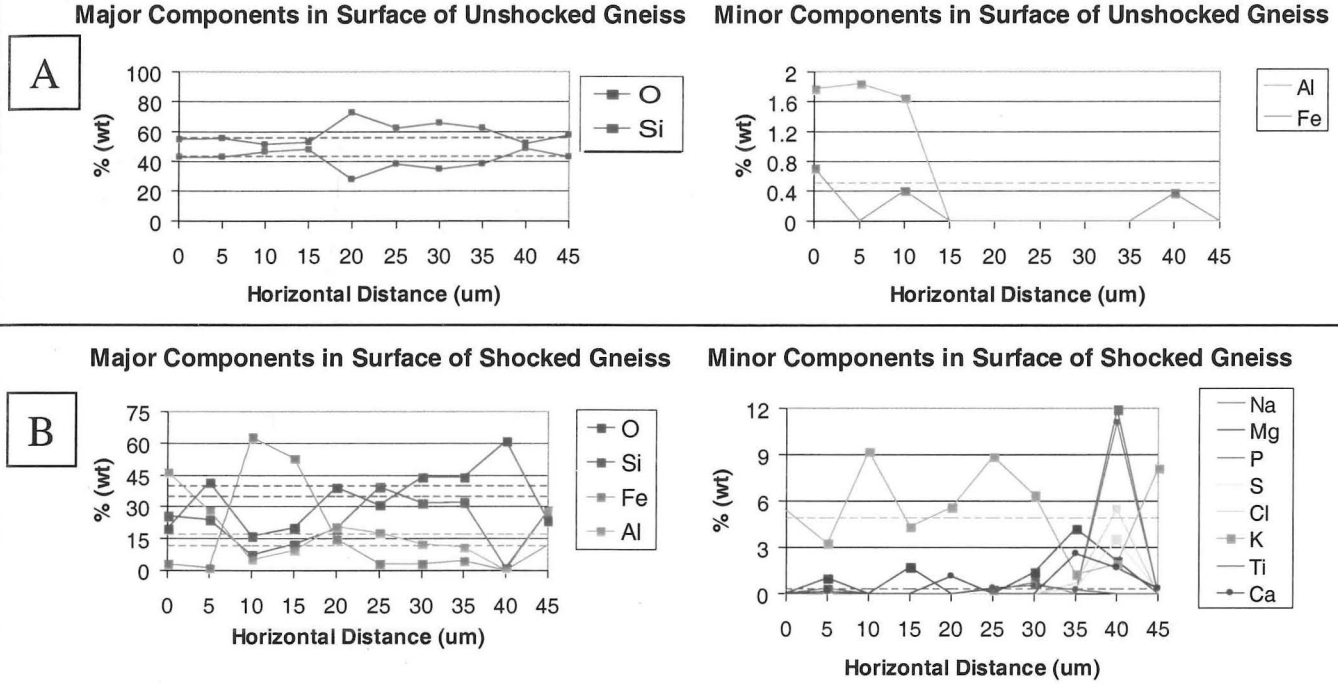

Figure 8: EDX Transects across surfaces. Transects consisted of 10 points, each sample a surface of 1 μm^2, separated by 5 μm. Dashed lines correspond to the average composition of an element over an area of 10^4 μm^2, containing the transect. Where no average value is shown, that element was not detected. (a) Unshocked gneiss. (b) Shocked Gneiss.

Overall, the surface composition as determined by EDX shows a remarkable disparity as compared with the bulk composition identified by ICP-AES. The ICP-AES bulk composition indicated there were significantly higher concentrations of Fe_2O_3, Al_2O_3, MgO, K_2O, Na_2O, TiO_2, and P_2O_5, as well as of all trace elements, in samples of unshocked gneiss. EDX spectra revealed the surface composition of unshocked gneiss to be composed almost entirely of silicon, oxygen, iron, and aluminium, whereas the shocked samples had surface compositions containing less iron and aluminium and significant amounts of calcium, magnesium, phosphorus, potassium, sulfur, and chlorine. These differences will be discussed at greater length in the next section.

Chapter 4
Discussion

Geological Interpretations

An examination of the composition of gneiss based upon the degree to which they have been shocked, yields insights about how the shocking process affects the composition of shocked gneiss compared to that of unshocked gneiss. Here, shocked samples have densities over 50% less than the unshocked samples, and porosities that are over an order of magnitude greater than the unshocked samples. The bulk chemical analysis using ICP-AES revealed the concentration of silica in shocked rocks is 10% higher than in unshocked rocks, with associated decreases in the concentrations of other oxides (primarily Fe_2O_3, Al_2O_3, and P_2O_5), although the concentration of calcium oxide remained roughly constant in the shocked samples. EDX analyses revealed the surface of the unshocked samples to be composed almost entirely of silicon, iron, and aluminium oxides, with trace amounts of titanium oxide, whereas the surface of shocked samples showed a higher degree of variability, especially within or adjacent to shock-induced vesicles, with observable concentrations of calcium, magnesium, phosphorus, potassium, sulfur, and chlorine.

We suggest that the disparity between the ICP-AES bulk composition data and the EDX surface composition data for the samples of unshocked and shocked gneiss can be explained as a result of the processes associated with the impact-induced shock metamorphism. First, as the shockwave resulting from the impact propagates through samples, darker minerals are preferentially volatilized because they absorb a greater fraction of the energy incident upon them. This volatilization would remove darker metallic oxides (particularly Fe_2O_3 and Al_2O_3) from the surface of rocks and create vesicles and fissures as near- and sub-surface localizations of these minerals volatilized. We suggest, furthermore, that, as the next category of minerals (the magnesium, phosphorus, potassium, and sodium oxides) volatilized, they left behind thin and/or patchy coatings on the interior of these vesicles. This would explain the presence of these latter minerals, localized around vesicles on the surface of the shocked samples (when they are absent from the surface of unshocked samples), despite the actual bulk percentage of these minerals being significantly less in shocked samples than in unshocked samples. The relative bulk abundance of biologically important elements such as sulfur, calcium, and chlorine, was not able

to be determined between unshocked and shocked gneiss because the ICP-AES analysis was not sensitive to them. However, it is likely that such nutrients are deposited from meltwater into impact-generated cavities, which would encourage endolithic heterotrophic bacteria to inhabit them.

Biological Interpretations

Comparison with Other Polar Heterotrophic Communities

The heterotrophic bacteria isolated from samples of shocked gneiss and breccia from Haughton impact structure are consistent with other heterotrophic communities identified from polar environments. Delille (1992) isolated 116 heterotrophs from marine and sea ice samples. Of these, 75% were Gram-negative rods belonging to the family *Pseuodomonadaceae* (35% of our identified isolates); the remainder were from the following genera: *Vibrio*, *Micrococcus*, *Arthrobacter* (25% of our identified isolates) and *Bacillus* (5% of our identified isolates).

The phylogenetic distribution of isolated bacteria from the samples of shocked gneiss and the breccia show a high degree of similarity to heterotrophic bacteria isolated from Antarctic sea ice, which Bowman *et al.* (1997) concluded represented "a rich source of psychrophilic, heterotrophic bacterial biodiversity." From the sea ice, Bowman *et al.* (1997) isolated primarily *Proteobacteria* (mostly γ and α), *Actinobacteria*, and *Firmicutes*, in addition to some members of the *Flexibacter-Bacteriodes-Cytophaga* phylum from Antarctic sea ice. For comparison, 13 *Proteobacteria* (11 γ; 1 α; and 1 β), 5 *Actinobacteria*, and 2 *Firmicutes* were isolated and identified from our samples of shocked gneiss and breccia. Specifically, shared genera between the two studies include *Pseudomonas*, *Arthrobacter*, and *Planococcus*, including two species found in both studies: *A. nicotiana*, and *P. citreus* (Bowman *et al.*, 1997).

The similarity between our isolates and the Antarctic sea ice isolates described by Bowman *et al.* (1997) is surprising when the different environments from which the bacteria were isolated is considered, but such similarity is not entirely without precedent: species of the phototrophic bacterium *Chroococcidiopsis* have been found as an endolith in both Arctic and Antarctic rocks (Cockell *et al.*, 2002); and a variety of heterotrophic gas vacuolate bacteria have been isolated from both sea ice and marine samples from the Arctic and Antarctica (Gosink & Staley, 1995). These heterotrophic bacteria belong to members of the phylogenetic classes α-, β-, and γ-*Proteobacteria* and the *Flavobacteria-Cytophaga* group, and also show high similarity to our

samples (Gosink & Staley, 1995). Furthermore, *Vibrio* species, the most common (cultivatable) heterotrophic psychrophilic marine bacteria (Wiebe *et al.*, 1992), have been found to be major components of Antarctic endolithic communities (Colwell *et al.*, 1989), and constitute more than 25% of the strains isolated from the sea ice (Delille, 1992).

Lacustrine environments also host a variety of psychrotrophic and psychrophilic heterotrophic bacteria. Saline lakes of the Vestfold Hills region of Antarctica were dominated by Gram-negative, non-fermentative rods, mostly *Pseudomonas spp.* (82%), the majority of which were psychrotrophic although there were a small proportion of psychrophilic isolates (Nichols *et al.*, 1995). An analysis of the heterotrophic community at Burton Lake revealed that it was dominated by psychrophiles (46% of 68 cultivated strains were unable to grow at 25°C), including isolates of *Flectobacillus*, *Flavobacterium*, *Pseudomonas* (35% of our identified isolates), and *Moraxella* (Nichols *et al.*, 1995).

Heterotrophic bacteria similar to the samples presented in this study have also been isolated from Antarctic soils. Aerobic heterotrophs within samples of ornithogenic soil number between 10^6 – 10^8 colony-forming-units per gram of dry-weight soil (Roser *et al.*, 1983). These soils harbor high populations of certain bacterial species, in particular *Psychrobacter immobilis*, nonhalophilic *Planococcus* (5% of our identified isolates) strains, and *Arthrobacter* (25% or our identified isolates) strains, which have also been isolated from sea ice and are probably transported into the ocean during the summer glacial melt (Bowman *et al.*, 1997).

The question remains as to the ultimate origin of these species, whether these organisms originated in ice or marine environments (and where subsequently transported to soil, from which they could have then colonized our samples), or they originated in soil and were carried via snowmelt and/or atmospheric circulation to ice or marine environments from which they were isolated. The ready transport of soil bacteria to ice has been demonstrated by studies of Antarctic ornithogenic soils (Bowman *et al*, 1997; Roser *et al.*, 1993; Rotert *et al.*, 1993, Delille, 1990, 1987). Similarly, studies have documented ice bacteria isolated from soil samples (Bowman *et al.*, 1997; Roser *et al.*, 1993). It is clear that further studies are necessary to identify the environments to which these bacteria are endemic. Additionally, it must be considered that the organisms may be naturally cosmopolitan, possessing a global distribution, rather than geographically limited to a certain area.

Origin of Isolates

The identification of the bacterial isolates from the shocked gneiss reveals that the majority are species associated with soil, ice, or freshwater, including a number that are also found in Antarctic soils and/or ice. Moreover, the common occurrence of genera and even species in ecologically and geographically diverse locations supports the notion of global transport and distribution of bacteria, by atmospheric circulation, meltwater flow, and animal vectors. Atmospheric circulation is most likely responsible for the long-distance transport necessary for the original deposition of the bacteria in the Haughton area (or, if the bacteria were originally endemic to Haughton, the likely mechanism for transporting the bacteria to the Antarctic). As mentioned earlier, precipitation, wind, and snowmelt have been identified as means of inoculating rock interiors with bacteria from the surrounding environment, and it is believed that a combination of these three factors is responsible for delivering the isolated bacteria to the interior of our samples.

The similarity of the bacteria isolated from within the breccia to those from the shocked gneiss, and to those isolated from Antarctic soil, strengthens the argument that the bacteria inhabiting these rocks are derived from the surrounding soil. It is estimated that $3 - 4 \times 10^6$ colony-forming units of heterotrophic bacteria inhabit the soil in the area from which the samples were collected. While the argument could be made that the bacteria are simply evidence of surface contamination in the process of extracting the interior fragments, thereby explaining both the presence of complex heterotrophic communities in the interior of these rocks and the similarities between the bacteria inhabiting the breccia and the shocked gneiss, we believe this not to be the case for several reasons. First, careful attention was given to selecting interior fragments to be used for obtaining the isolates. The original rocks (approximately 10 cm in diameter) were broken into several centimeter-size pieces, from which only fragments showing fresh break marks and with no evidence of surface weathering were chosen. These fragments were then broken open again, with millimeter or sub-millimeter fragments from the center used to isolate bacteria from the samples. During the isolation process, growth was observed to occur in the Petri dish only in those areas that surrounded these fragments. As a result, the likelihood that surface materials contaminated these fragments is deemed to be insignificant. Perhaps a stronger argument against surface contamination would be the lack of phototrophic organisms isolated in our process. The outer surfaces of both shocked gneiss and breccia are observed to

have visible coatings of cyanobacteria and other photosynthetic organisms. Further, Cockell *et al.* (2002) identified endolithic phototrophs living in the upper few millimeters of similar rocks. Phototrophic bacteria would be present in any surface and near-surface contamination and their complete absence in the isolates obtained from both the shocked gneiss and breccia samples indicates that surface contamination could not have had a significant impact upon our results.

Assessment of SEM/EDX for *In Situ* Identification of Endolithic Microorganisms

Isolation and identification of bacteria from samples of shocked gneisses from Haughton Impact structure reveals that a community of heterotrophic bacteria (predominantly chemoorganotrophic, but with the possibility of chemolithotrophic species of *Bacillus* and *Pseudomonas*) inhabits the interior of these rocks. Examination of interior fragments of shocked gneiss by SEM microscopy revealed the presence of both coccoid and rod-shaped bacteria inhabiting cavities within the rock. Application of EDX to the surface of these fragments verified the biogenicity of these putative bacteria by demonstrating a distinctly biological signature in those cavities, in which the SEM images show colonies of bacteria. Its application, on the other hand, to the surface *distant from cavities* reveals the surface composition to be non-biological (primarily silicon, aluminium, and iron oxides). A comparison of the elemental spectra taken from the microbe-inhabited cavities with the spectra obtained from bacterial isolate G20 (isolated from the interior of shocked gneiss) on a glass slide show a high degree of similarity, both in the appearance of biologically important elements and in the relative strengths of their peaks. The presence of chlorine appears to be the strongest diagnostic indicating the presence of life. Also, the presence of sulfur, phosphorus, and the ratio of potassium to calcium appear to indicate the presence of biological organisms. We demonstrate that the combination of SEM and EDX, suggested by Wierzchos & Ascaso (2002) as a tool for identifying microfossils, is a valid technique for the identification of heterotrophic endoliths.

Relevance to Meteorites

The presence of microfossils in the surface and near-surface environments of meteorites clearly demonstrates their exposure to terrestrial contamination. An examination of shocked rocks from the Haughton impact structure revealed the efficacy with which their interiors have been colonized by heterotrophic bacteria. The prime factor facilitating this colonization appears

to be the impact-induced porosity of the shocked rocks. The interior of meteorites experience impact shock at pressures far in excess of the samples examined in this study, which should strongly favor the heterotrophic colonization of meteoritic interiors. The source for such contamination could be either deposition from atmospheric circulation (the source of the microfossil contamination previously studied) or, more likely, the ice immediately surrounding the meteorite. It has been observed that the Antarctic ice sheet contains a variety of heterotrophic bacteria (Franzmann, 1996; Kellogg & Kellogg, 1996; Hirsh *et al.*, 1988). Further, the albedo difference between the meteorite and the surrounding ice, would cause the surrounding ice to melt when the meteorite is heated by solar radiation during the Antarctic summer. This would concentrate any heterotrophs from the nearby ice into a pool of meltwater surrounding the meteorite, providing a mechanism to inoculate the interior of the meteorite with heterotrophs.

Life on Mars

The observed impact-induced generation of habitats in crystalline rocks also has clear relevance to astrobiological questions of life on the surface of other planetary bodies with impact-shocked surface materials. This is particularly true of Mars, where as a result of the lack of global plate tectonics, the age of exposed surface materials can exceed 3.9 billion years, a time when the impact rate was significantly higher than its present value. Were life to have evolved on Mars, impact-shocked rocks would have provided ready lithic habitats, without the required wait for the creation and exposure of sedimentary rocks. Further, as Mars cooled and water became increasingly scarce, the interior of impact-shocked rocks could have served as a reserve for water, heat, and nutrients for any Martian organisms.

Chapter 5
Conclusions and Significance

Summary

An examination of the physical characteristics of unshocked and shocked gneiss clearly demonstrates that impact-induced shock metamorphosis creates new habitats for microbial life to exploit. This is observed most clearly by the presence of impacted-induced vesicles in the shocked gneiss samples, which provide the physical environment that the bacteria may inhabit. Furthermore, the surfaces of these vesicles are enriched in biologically important elements, providing the necessary chemical environment for the bacteria to thrive. The isolation of 14 heterotrophic bacteria from the interior of samples of shocked gneiss reveals the efficacy with which bacteria have taken advantage of these impact-generated habitats. The isolation of an additional 13 heterotrophic bacteria from the interior of breccia samples suggests that microbial habitats were created across the entire region of influence of the impact event. Finally, the similarity of the bacteria isolated from shocked gneiss and the breccia, both to themselves and to isolates from Antarctic soil, indicates that the likely source of the bacteria colonizing these shocked rocks is the soil in the immediate vicinity, and that long distance transport, most likely due to atmospheric circulation was responsible for the distribution of the bacteria. A combination of SEM and EDX observations demonstrate the *in situ* growth of these bacteria on the interior of the samples, and is suggested as a technique for the identification of organisms within such samples, with particular emphasis on the presence of chlorine as an indication of biological activity.

These data point to the existence of a significant community of endolithic, heterotrophic bacteria growing *in situ* within the interior of samples of shocked gneiss. The location of these endoliths appears to be dependent not only on impact-induced porosity, but also on impact-induced inhomogeneities in surface mineralogy, particularly the abundance of biologically important elements found within surface cavities, that occur within samples of shocked gneiss from the Haughton impact structure. While prior work has documented phototrophic microorganisms in the near surface region of impact-shocked crystalline rocks (Cockell *et al.* 2002), this is the first documentation of the colonization of the interior of impact-shocked rocks by heterotrophic bacteria. The discovery of these heterotrophic communities within impact-

shocked crystalline rocks extends our knowledge of the habitable biosphere on Earth. It also has particular relevance to meteorites recovered from the Antarctic ice sheet, where long-term surface exposure may be sufficient for bacteria in the surrounding ice to colonize the interior of these meteorites, particularly in light of recent work showing the variety of organisms isolated from Antarctic ice and the efficacy of wind, precipitation, and snowmelt as mechanisms for transporting organisms. Furthermore, the demonstration that impact events generate abundant microbial habitats has applications to the origin and survival of life on planetary bodies, most particularly Mars, with exposed surfaces of impact-shocked materials.

Future Work

There are several more avenues of analysis that could be investigated to increase our understanding of the nature, extent, and origin of the heterotrophic colonization of impact-shocked rocks from Haughton impact structure. First, it would be insightful to isolate heterotrophic bacteria from the interior of unshocked samples. Although the likelihood of this isolation is deemed low, it would be valuable to identify any bacteria that live inside unshocked samples and to ascertain how the bacteria were transported there. A thorough characterization of the isolates obtained from the shocked gneiss and breccia, particularly their metabolic and physiological characteristics, would add depth to the analysis of the origins of these bacteria and their interactions with the endolithic environment. It would be of particular interest to try to isolate bacteria on a media containing different nutrients in different quantities and at different temperatures from 4°C to 30°C. It would be useful to conduct PCR directly on interior fragments of shocked gneiss and breccia. This would identify all of the microorganisms within these samples, whereas only approximately 1% of all species are able to be cultured in a laboratory (and thus, the isolates identified in this study represent only a small portion of those present in the samples). This would provide a more thorough characterization of the community structure in the interior of these rocks. However, conducting PCR directly on environmental samples without isolating bacteria, raises the possibility that species identified may not have been living or viable within the samples, because PCR can amplify rRNA of dead microorganisms, in addition to that from those living. These analyses would provide a more thorough understanding of the endolithic heterotrophic communities inhabiting the interior of the shocked crystalline rocks.

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